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Modeling a Reversed β-oxidation Cycle into the Genome Scale Model of *Zymomonas mobilis*

by

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ABSTRACT

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This study proposes simulations which present optimized methods for producing fatty acids, fatty alcohols and alkanes using *Zymomonas mobilis* bacterium by the energy efficient β-oxidation reversal pathway, an eco-friendly alternative to the present petroleum based processes. Zymomonas has advantages of higher carbon intake, higher ethanol tolerance and higher ethanol production efficiency than other organisms. I have improved an earlier Zymomonas genome scale model and used Constraint Based Reconstruction and Analysis (COBRA), a linear optimization based computational tool in Matlab, and to perform flux balance analysis (FBA) based simulations. FBA accounts for formation, consumption, accumulation and removal rate or flux of each metabolite. The results present ***solution spaces*** of cell growth rate and product formation rate, which trend with products and their carbon chain length. I have analyzed these ***solution space*** trends gaining insight into the Zymomonas' metabolism, enabling efficient product formation and opening a way for future improvement.

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Nomenclature

COBRA	Constraint Based Reconstruction and Analysis
FBA	Flux balance analysis
ATP	Adenosine triphosphate
NADH	Nicotinamide adenine dinucleotide
GL	Glycerol
DHACT	Dihydroxyacetone
T3P2	Dihydroxyacetone Phosphate
DOROA	(S)-Dihydroorotate
OROA	Orotate
METTHF	5, 10-Methylenetetra-hydrofolate
MTHF	5, 10-Methenyltetra-hydrofolate
Q	Ubiquinone
QH_2	Ubiquinol
HEXT	External proton
H	Internal proton
ccfe2	cytochrome c (FeII)
ccfe3	cytochrome c (FeIII)

Chapter 1

Introduction

This thesis proposes simulations, which present optimized methods for producing fatty acids, fatty alcohols and alkanes using *Zymomonas mobilis* bacterium by the energy efficient β-oxidation reversal pathway, an eco-friendly alternative to the present petroleum based processes. I have improved a Zymomonas genome scale model developed by Lee et al. and used Constraint Based Reconstruction and Analysis (COBRA), a linear optimization based computational tool in Matlab, to perform flux balance analysis (FBA) based simulations. I have analyzed the obtained results gaining insight into the Zymomonas' metabolism. The results and analysis from this study quantitatively present an efficient product formation strategy and opens the way for future improvement of Zymomonas by genetic manipulation study using the modified model.

1.1. Motivation

Eco-friendly processes have become the need of the hour. With the rising pollution from various industrial processes one gets concerned about planet earth. Increasing awareness has led us into several innovative sources and processes that satisfy the need of a healthy & pollution free environment. In this study I have described an eco-friendly method of producing several useful products which touch our lives in many ways. The method is using a bacteria *Zymomonas mobilis,* which has been shown in this study to produce a range of products such as fatty acids, fatty alcohols and alkanes using carbon sources such as glucose, glycerol and xylose. This has been shown to be possible in this study by performing simulations on a modified genome scale model of *Zymomonas mobilis* ZM4.

Simulations play a major role in the field of metabolic engineering. Metabolic engineering has two components- synthesis and analysis. A design strategy for the synthesis process is implemented, based on an objective to improve cell growth or product formation or both; the results are then analyzed. Design strategy is revised following on the results' analysis, to achieve the initial objective and the new results are analyzed again. This cyclic process of synthesis design and analysis thereof goes on till the initial objective is met with(Stephanopoulos 1994). Simulations suggest implementation of such optimum design strategies to meet the objective. Simulations further predict results, enabling initial analysis of the design strategy which can be verified by experiments. Simulations are faster and effective methods of analyzing workings of any system. The faster results and lesser resource

requirements of theoretical simulations, as compared to experiment results, make them an easy starting choice to tackle a problem.

The simulations in this study are performed on a genome scale model by Lee et al.(Kyung Yun Lee, Jong Myoung Park, Tae Yong Kim, et al. 2010). I have modified the model by introducing certain changes and additions. The modifications are on account of certain problems I faced while working with the original model which have been explained in detail in materials and methods chapter. Also, I have added the β-oxidation reversal pathway, an energy efficient pathway compared to the fatty acid synthesis pathway, to produce the desired products. The various steps of the β-oxidation reversal pathway as used in the simulations have been discussed further in materials and methods chapter.

These simulations have been performed on Matlab R2010a by using a linear optimization based computational tool Constraint Based Reconstruction and Analysis (COBRA) v2.0. COBRA uses the underlying concept of flux balance analysis (FBA) which takes into account formation, consumption, accumulation and removal rate or flux of each metabolite to arrive at an optimized solution. In this study the results, generated using COBRA, present ***solution spaces*** of cell growth rate and desired product formation rate after the removal of other undesired metabolites. Analysis of the trends in these ***solution spaces*** which vary with products and carbon chain length reveal more insight into the organism's metabolism and helps in further genetic manipulation. As mentioned before the organism considered in this model is *Zymomonas mobilis* which has been discussed in the following section.

1.2. Zymomonas

One of fascinating bacteria in the microbial world, Zymomonas was notorious for causing “cider-sickness”. It spoiled the taste of sweet ciders by causing secondary fermentation. Zymomonas was first purified from the complex micro flora in the spoiled cider. The identification of especially motile strains led to the specific name mobilis. Zymomonas is still used to produce palm wine in several parts of the world by fermenting palm sap. Therapeutic uses of Zymomonas has also been reported on several occasions (Swings and De Ley 1977). Zymomonas is known for its high specific sugar uptake rate and high ethanol production rate. It also has a higher ethanol tolerance than other organisms (Seo et al. 2005). These properties make it an ideal choice for fermenting various carbon sources. Zymomonas uses the Entner-Doudoroff pathway for fermentation which is described in the following section.

1.2.1. Entner-Doudoroff pathway

The Entner-Doudoroff pathway is the main carbon metabolism pathway in Zymomonas. The Entner-Doudoroff pathway was first discovered in *Pseudomonas saccharophilia*. It has been claimed to be older than the more conventional glycolytic pathway, Embden-Meyerhof-Parnas glycolysis, prevalent in most organisms (Conway 1992). The pathway has been seen in several other organisms and operates in several modes across organisms. Inducible mode of the Entner-Doudoroff pathway exists in *E.coli,* where gluconate acts as an inducer to activate the pathway. Cyclic mode of the pathway operates in *P.aeruginosa,* where one of the

intermediates glyceraldehyde-3-phosphate eventually gets converted back into glucose-6-phosphate and vice-versa. In case of *Zymomonas mobilis*, the Entner-Doudoroff pathway operates in a linear way. The key features of this pathway as shown in Figure 1.1 include the formation of 6 carbon intermediate 2-keto-3-deoxy-6-phosphogluconate (KDPG) from glucose by consuming 1 ATP and generating 1NADPH as reducing equivalent.

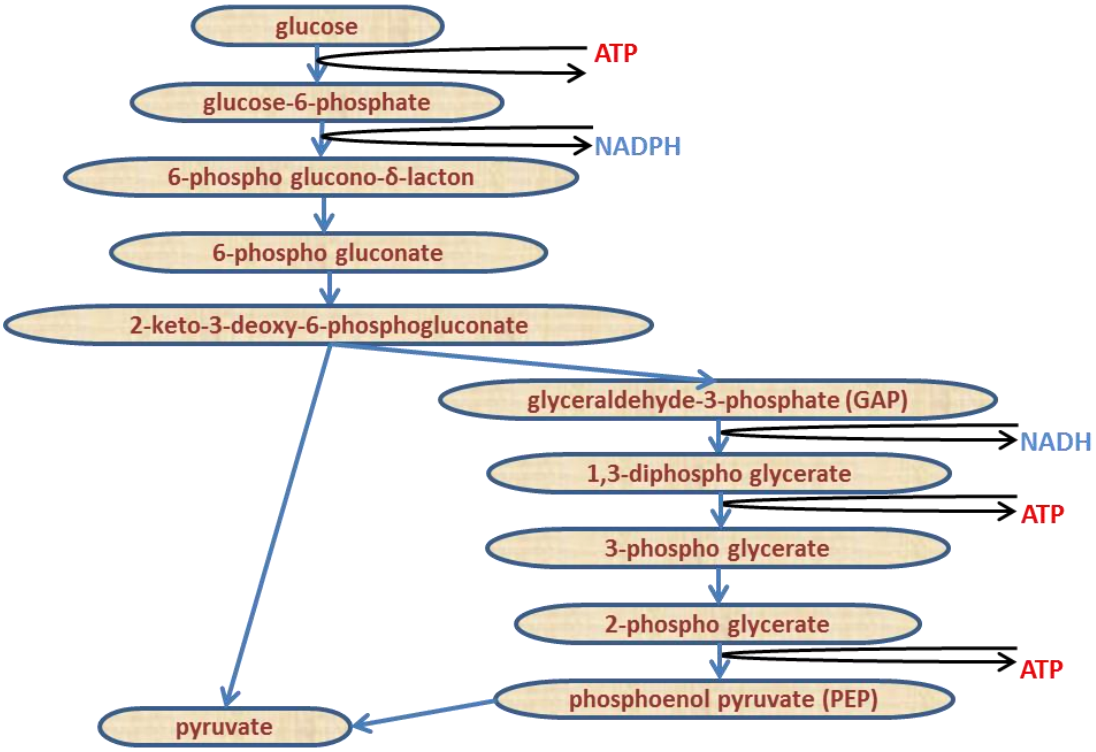


Figure 1.1 - The Entner-Doudoroff pathway in *Zymomonas mobilis* showing the conversion of glucose to pyruvate with reducing equivalents and ATP.

KDPG then splits into pyruvate and another 3 carbon intermediate glyceraldehyde-3-phosphate (GAP). GAP subsequently also gets converted to pyruvate, generating 2 ATP and 1 NADH during the conversion. Thus, over all from

glucose to pyruvate the pathway generates 1 ATP, 1NADPH and 1 NADH. This can be compared to the more common EMP pathway found in most organisms, which generates 2 ATP and 2 NADH while converting glucose to pyruvate. As a consequence of this there is more biomass or cell growth observed in case of the EMP pathway but the product yield is more in case of the ED pathway.(Bai, Anderson, and Moo-Young 2008)

The importance of the Entner-Doudoroff pathway have been realized in this study, as the flux balance analysis in the discussion chapter reveal the active presence of this pathway. This pathway occurs in all strains of Zymomonas. Of the various Zymomonas strains, ZM4 has been considered in this study.

1.2.2. ZM4 strain

ZM4 strain has been shown to be superior over other Zymomonas strains in terms of rate of ethanol production, cell growth, glucose uptake, and ethanol tolerance. Experimental comparison of ZM4 strain with other strains has been done by Lee et al to corroborate the above statement (K.J. Lee, Skotnicki, Tribe, et al. 1980). ZM4 strain lacks two enzymes 2-oxoglutarate dehydrogenase and malate dehydrogenase. It however is still able to produce the necessary intermediates through alternative pathways which have been considered in the genome scale model by Lee et al.(Kyung Yun Lee, Jong Myoung Park, Tae Yong Kim, Yun, and Sang Yup Lee 2010). The study proposes methods of optimizing the Zymomonas ZM4 strain to generate various products.

1.3. Desired Products

There are three main groups of desired products which have been discussed in this study – fatty acids, fatty alcohols and alkanes. Fatty acids find use in diversified industries. They are used in manufacturing soaps, detergents, paints, cosmetics, food products and several others as starting materials or in the form of derivatives. Fatty acids are also used to manufacture insecticides, disinfectants and germicides.

Fatty alcohols and their derivatives also find use in the industry such as polymer processing and paper processing. Fatty alcohols find unconventional use as tobacco sucker agents, to increase the quality and yield of tobacco. Fatty alcohols are used sometimes to prevent evaporation by forming a layer of coating over water reservoirs in dry seasons.

Alkanes find their utility as Commercial fuels and as components of gasoline. In addition alkanes are also used in polymer manufacturing and as intermediates in manufacturing of various chemicals such as ethanol and acetic acid. Other uses include as paraffin wax and plant hormone such as ethylene used to ripen fruits.

These high utility products are currently being produced at industrial scale from petroleum sources. This study proposes the production of the aforementioned products as descried in the following objective section.

1.4. Objective

The main objectives of this proposed study can be delineates as follows.

- To simulate production of fatty acids, fatty alcohols and alkanes using β-oxidation reversal cycle in *Zymomonas mobilis* ZM4.
- To optimize the production of desired product by simulating removal competing metabolites.
- To compare between different carbon sources- glucose, glycerol and xylose/arabinose based on simulations of their ability to produce the desired product.

This study proposes simulation of *Zymomonas mobilis* ZM4 genome scale model to produce various fatty acids, fatty alcohols and alkanes. This study proposes several modifications on the existing Zymomonas genome scale model to obtain better results. The β-oxidation reversal cycle is the pathway used in this study to achieve the production. This study suggests optimized production by following simulation results after removal of competing metabolites. This study also draws out comparison between three different carbon sources – glucose, glycerol and xylose/arabinose by comparing model simulations with each of them exclusively. Yield and productivity analysis of the products bring out the comparison between the different carbon sources. The following chapter describes how this has been achieved.

Chapter 2

Materials and Methods

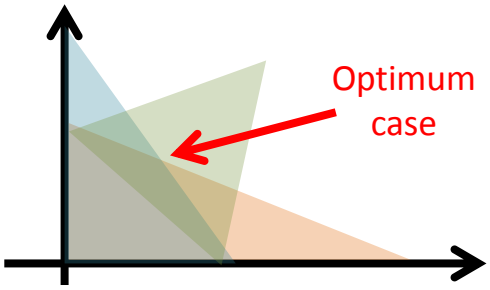
This study uses genome scale model to perform simulations on. I have used Constraint –Based-Reconstruction and Analysis (COBRA v2.0) toolbox in MATLAB R2010a, which further uses flux balance analysis (FBA) to perform constraint based linear optimization to arrive at solutions. This chapter describes the above mentioned methods and model used in the study.

2.1. Flux Balance Analysis (FBA)

Orth et al. describe flux balance analysis (FBA) as a mathematical approach based optimization of a set of biochemical network of reactions subjected to mass balance constraints and helps predict growth rate and product formation rate(Orth, Thiele, and Palsson 2010). FBA is used to analyze metabolic networks and hence quite useful in case of genome scale models.

Consider a set of three reactions R_1, R_2 and R_3 comprised of a set of 10 metabolites m_i (i=1...10). These can be represented as follows in form of a matrix based on the stoichiometric co-efficient of each metabolite represented as the matrix elements where each row represents a metabolite and each column represents a reaction.

$$m_1 + m_2 \rightarrow m_3 + m_4 \cdots\cdots\cdots\cdots\cdots R_1$$
$$m_4 + m_5 \rightarrow m_6 + m_7 \cdots\cdots\cdots\cdots\cdots R_2$$
$$m_8 + m_9 \rightarrow 2m_3 + m_{10} \cdots\cdots\cdots\cdots\cdots R_3$$



	R_1	R_2	R_3
m_1	-1	0	0
m_2	-1	0	0
m_3	1	0	2
m_4	1	-1	0
m_5	0	-1	0
m_6	0	1	0
m_7	0	1	0
m_8	0	0	-1
m_9	0	0	-1
m_{10}	0	0	1

Assuming Pseudo steady state

$$rate\ of m_3\ formation = rate\ of\ reaction\ R_1 + 2 * rate\ of\ reaction\ R_3 = 0$$

Figure 2.1 – Matrix representation of a set of reactions where each row represents a metabolite, each coloumn represents a reaction and each matrix element represents the stoichiometric co-efficient of the corresponsing metabolite in the corresponding reaction. This is followed by a linear optimization based analysis to obtain optimized reaction rates or flux values.The various shaded regions in the bottom left figure represent constraints.

The flux values or rates of the above mentioned reactions are bounded by maximum and minimum values and then linear optimized to maximize growth rate, represented by biomass, subjected to mass balance constraints and obtain optimum flux values. Biomass is also accounted for by a hypothetical reaction with

metabolites, those reported to contribute to cell growth and the stoichiometric co-efficient based on their relative contribution, verified by experimental results. Reactions to be removed are subjected to maximum and minimum flux values of 0. I have imposed these steps on the Zymomonas genome scale model, described latter. I have used a MATLAB toolbox called Constraint –Based-Reconstruction and Analysis (COBRA) to implement flux balance analysis (Schellenberger et al. 2011).

2.2. COBRA

Constraint –Based-Reconstruction and Analysis (COBRA) is a MATLAB based toolbox which deals with systems biology analytical tools. It has been used to develop several methods and analyze several things such as Transcriptomic analysis, Signaling networks, Host-pathogen interactions, Metabolic engineering (Schellenberger et al. 2011). I have used the flux balance analysis tool to obtain optimum flux values for various reactions in the genome scale model. I have added the genome scale model reactions from work by Lee et al. with respective flux bounds to a MATLAB program followed by further addition of reactions and pathways such as the β-oxidation reversal pathway. I have also performed Flux variability analysis, a method to obtain the maximum and minimum flux values while maintaining the optimum value constant, on the modified model to obtain ***solution spaces*** which are discussed in the results chapter. The pathway and the genome scale model are discussed in the following sections.

2.3. β-oxidation reversal pathway

The β-Oxidation pathway consists of a set of reactions which metabolize fatty acids. The reversal of the pathway has been reported to have successfully and energetically favorably produced fatty acids and derived products(Dellomonaco, Clomburg, Elliot N Miller, et al. 2011). There are four different steps in the cycle as shown in Figure 2.2. The first being conversion of acyl-CoA to hydroxy acyl-CoA. The next being conversion of hydroxy acyl-CoA to enoic acyl-CoA, followed by conversion of enoic acyl-CoA to oxo acyl-CoA and finally conversion of oxo-acyl CoA to acyl-CoA to complete the cycle. Termination at each step produces varied substituted products with groups such as hydroxy, enoic or keto.

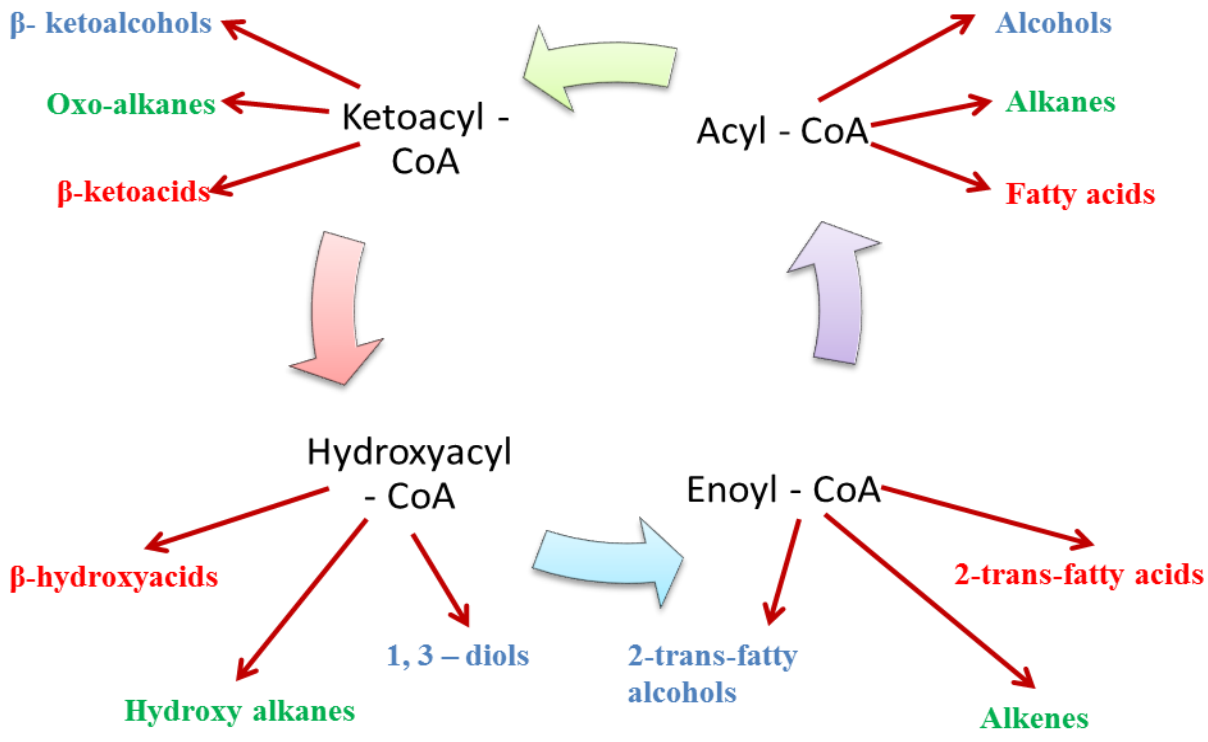


Figure 2.2 – Four steps of β-oxidation cycle reversal and corresponding products from each step.

Varying the termination causing enzymes leads to production of alcohols and alkanes besides acids. The aforementioned spectrum of products is considered in this study by the adding the above pathway to the genome scale model by Lee et al.

2.4. Genome Scale model

With the advent of high throughput technologies large amount of systemic data can be processed. Next follows the use of gathered information from the processed data to gain more insight into the system under consideration. In case of bacteria we have a lot of information about the various biochemical reactions taking place inside the cell. It consists of several pathways which have been studied or explored independently. The idea behind genome scale model is to bring together all these different pathways and look at the conglomerate on a genome scale.

A Zymomonas genome scale model has been constructed by Lee et al. using different software and databases, which include “NCBI, CMR, KEGG, TCDB, TransportDB, ExPASy, BioCyc, Bio- Silico, MFAML, and MetaFluxNet.” The model consisted of 579 metabolites and 601 reactions which accounts for 347 open reading frames (ORFs) with a 20.1% over-all coverage, comparable to other existing genome scale models(Kyung Yun Lee, Jong Myoung Park, Tae Yong Kim, Yun, and Sang Yup Lee 2010).

Another Zymomonas genome scale model was constructed by Widiastuti et al. based on open reading frame (ORF) information from previous works on Zymomonas genome. The information has been collected from KEGG and BioCyc

databases to construct the model with various reactions and metabolites. The model hence created contained 704 metabolites and 747 reactions and accounted for 363 ORFs (Widiastuti et al. 2011). Both the models were similar in their coverage of genomic information, hence either could be chosen to perform simulations on with similar accuracy in prediction. I have chosen the former model created by Lee et al. for my study.

Metabolic production rates and reaction rates reveal qualitative information about the metabolic state of the cell represented by the corresponding metabolites and reactions. Under the constraints of mass balance, assuming equilibrium to be maintained all metabolites have fixed concentrations with the total rate of production equal to the total rate of consumption(Durot, Bourguignon, and Schachter 2009). This assumption provides a law of mass conservation perspective which when applied to the model could reveal missing information about the model. This missing or 'gap-filling' information provide information which may not have been verified but can be inferred from the mass balance necessity. Thus the process of reconstruction and validation of such a model has its own utility.

However, the kinetic parameters of various enzymes impose limitations on the scope of the model because of the complex mathematical relations and unknown parameters involved in the actual biochemical reactions. This restricts the model to a portion of what represents the actual metabolic network. Constraints are applied on the models, based on thermodynamic and mass balance feasibility, to account for the limitations to some extent. The thermodynamic constraint ensures the

reversibility of reaction while the mas balance constraint in addition with rate limitations of a reaction ensures limitation on possible flux values. Stoichiometric relations between metabolites are also considered in the model. Reaction fluxes are normalized reaction rates per unit weight of the cells, which accounts for variation in cell size.

2.4.1. Modifications

I have simulated the model on certain tests, as mentioned below to corroborate the results as referred to in the work by Lee at al. (Pinto, Dias, Lourenço, et al. 2009). This would set up an initial base line to build improvements into the model. The simulated theoretical yields of Ethanol and Succinate, I obtained by maximizing the simulated production of ethanol and succinate exclusively, matched closely the values mentioned by Lee et al. However, the simulated results showed large deviations while implementing genetic modifications, obtained by removing the reactions represented by the gene, suggested by Lee et al. to improve succinate production. I have improved and expanded the model to account for the discrepancy between the simulated and other previously reported results. The first modification to the model was addition of reaction bounds to the model, followed by modifications mentioned in the following sections.

2.4.1.1. Glycerol Metabolism

I have added the following reactions into the model to ensure glycerol intake and further metabolism by the model.

'GL + NAD → DHACT + NADH', (Asnis 1953)

'DHACT + ATP → T3P2 + ADP',(Sellinger, Z., and O. N. Miller. 1985)

Where GL – Glycerol; DHACT – Dihydroxyacetone; T3P2 – Dihydroxyacetone Phosphate

The following reaction was added to the model to act as redox sink to account for the reduced state of glycerol.

'GL + NADH → 13PDO + NAD',(Zeng 1996)

Where 13PDO – 1, 3-Propanediol

The simulated model, however, showed cell growth even in the absence of the above reaction which meant that there was consumption of the additional reducing equivalents (NADH) generated due to glycerol consumption elsewhere in the model.

2.4.1.2. NADH Problem

Upon analyzing the flux values I found that the model was using NAD as an external source. In other words, the model was able to uptake NAD freely and uses NAD to produce NADH. I removed this reaction.

The model now did not show growth under anaerobic conditions. Analyzing the fluxes I came across the following reaction representing L-aspartate oxidase.

'ASP + O_2 → ISUCC + H_2O_2' ,(Bossi, Negri, Tedeschi, et al. 2002)

Where ASP- Aspartate; O_2- Oxygen; ISUCC- Iminosuccinate; H_2O_2 – Hydrogen Peroxide

ISSUC is a precursor for producing NAD and the above reaction being the only source was inactive under anaerobic conditions. Looking up for an isomerase for Zymomonas which performed the above mentioned function of producing ISSUC didn't yield any results. So, I went ahead and added the following reaction to make fermentation feasible in the model which has already been experimentally established.

'ASP + FUM → ISUCC + SUCC',(Tedeschi et al. 2010)

Where FUM- Fumarate; SUCC – Succinate

Glycerol fermentation was now possible in the model. There was however still no PDO consumption which indicated that there were certain reactions consuming the extra NADH still. I found out that the problem with NADH consumption was due to these reactions:

'NADH + Q → NAD + QH_2 + 2 HEXT',

'QH_2 + *ccfe3* ←→ Q + *ccfe2* + 4 HEXT', (Wikstrom, Krab, and Saraste 1981)

'ATP ←→ ADP + PI + 2 HEXT',

Where Q – Ubiquinone; QH_2 – Ubiquinol; HEXT – External proton; *ccfe2* -cytochrome c (FeII); *ccfe3* - cytochrome c (FeIII)

The aforementioned reactions were leading to consumption of NADH and production of ATP which would not happen in presence of the other substrate and product in 2nd reaction. To fix this I have added the other substrate and product for the 2nd reaction, those being cytochrome c (FeIII) and cytochrome c (FeII). But the above metabolites are essential part of the electron transport chain hence not active under fermentative conditions. These metabolites were missing in the reaction present in the model making it constraint free. I added the following reaction to ensure balance of the metabolites and impose constraint on the previous reaction involving these metabolites.

'O_2 + 4 ccfe2 → 4 ccfe3 + 4 HEXT', (Vijgenboom, Busch, and Canters 1997)

The absence of the above modifications and additions was making the model consume NADH and prevent making any reduced metabolite, such as propanediol, from glycerol. After fixing this problem I faced problem due to $FADH_2$ being accumulated in the model and non-conversion into other reducing-equivalents such as NADH.

2.4.1.3. $FADH_2$ Problem

I added the reactions representing the four steps of the reversal β-oxidation cycle. I added termination reactions as per the following general structure to produce products from the cycle and the model worked with the following additions to produce alcohols.

'AcylCOA + NADH ←→ Acyl-aldehyde + NAD'

'Acyl-aldehyde + NADH ←→ Acyl-alcohol + NAD'

'Acyl-alcohol →'

I added and verified the termination reactions for alkane production from *Botryococcus braunii*. These reactions follow the following general structure.

'Acyl-aldehyde ←→ Alkane + CO',

'Alkane →'

The 4th step of β-oxidation reversal cycle could be represented by two sets of reactions. While one of them, FadE, used $FADH_2$ as reducing equivalent, the other set, egTER, used NADH as reducing equivalent. I added the reaction representing FadE. Upon adding the reaction for egTER the cycle didn't work. Upon further analysis of the problem I realized that the later set of reactions only worked in the presence of a reaction enabling conversion of FADH2 to NADH. Thus, there was an accumulation of $FADH_2$. Upon following the flux values I came across the following reaction which was consuming $FADH_2$. I verified with genomic information reported by Seo et al., that the reaction used NADPH instead of $FADH_2$ and I replaced it.

'METTHF + NADPH ←→ MTHF + NADP', (Seo et al. 2005)

Where METTHF - 5, 10-Methylenetetra-hydrofolate; MTHF - 5, 10-Methenyltetra-hydrofolate

The problem with FADH2 was partly resolved by removing the following reaction.

'FUM + QH_2 ←→ SUCC + Q'

The reaction was producing Succinate which was getting converted back into Fumarate and producing FADH2. Checking further for other coupled reactions I came across the following reaction in the model has been replaced in the KEGG database (EC 1.3.3.1).

'DOROA + Q ←→ QH_2 + OROA'

Where DOROA – (S)-Dihydroorotate; OROA – Orotate

The replacement of the above reaction solved the problem with not being able to replace FadE (reaction using NADH) with egTER (reaction using $FADH_2$).The above reaction was coupled via QH_2 to production of $FADH_2$, which was getting consumed only by FadE in absence of succinate; hence, making FadE irreplaceable with egTER. The reaction below when replaced the above reaction solved this problem by replacing ubiquinone and ubiquinol with fumarate and succinate respectively(Cheleski et al. 2010).

'DOROA + FUM ←→ SUCC + OROA'

The model showed more cell growth with FadE as compared to egTER because of the reaction which converted NADH into $FADH_2$. I have added the following reaction into the model to ensure exchange of reducing equivalents. The following reaction consumes internal proton, driving the proton pump to produce more ATP for the cell growth.

'FAD + NADH + H → $FADH_2$ + NAD'

2.4.1.4. ATP maintenance

Having fixed the problems with the model I needed to add fixed reaction bound to the ATP maintenance reaction to account for non-growth associated maintenance.

To find the ATP maintenance values those reported by several articles based on various ranges are considered. I considered the value of 16 $mmol\ ATP\ g^{-1}\ h^{-1}$ from following the article by Lee et al. (Wen-Chien Lee and Huang 2000). Their value had been calculated from 15 different data points making it more reliable. The inclusion of the fixed value for the maintenance reaction showed small cell growth (around 0.05 h^{-1}) or no growth for the case of genetic modifications, after removal of undesired products. Thus, I had to lower the value to ensure feasible cell growth. I referred to the work done by Sáez-miranda et al and have fixed the value of non-growth associated maintenance around 1.1 $mmol\ ATP\ g^{-1}\ h^{-1}$ assuming conditions of low pH and low acetate concentration as explained in the mentioned work (Sáez-miranda, Saliceti-piazza, and Mcmillan 2004).

2.4.1.5. Internal Protons

With the above modifications the model was showing cell growth of around 1.6 h^{-1} as compared to a value of around 0.4-0.5 h^{-1} found in literature. This section deals with this problem of such very high growth rate. The model does not include certain metabolites such as water (H_2O), internal protons (H), under the assumption that the mass balances of those are ensured by their presence as both substrates

and products across the model uniformly. Water has free transport across the cell, thus absence of water in the model does not affect the simulation results. The same is not the case with internal protons .To fix this I have added internal protons (H) to the whole model keeping the new modifications I had previously done to the model. Under wild type conditions the cell showed growth rate of 0.45 h^{-1}. Repeating the simulations for the suggested genetic modifications to increase succinate production I got results around and less than 0.4 h^{-1}, much closer to those reported. There was still some problem with the model observed as the ethanol yield was 0.36 as compared to the experimental evidence of around 1.9. This was fixed by rectifying the following reaction, adding an internal proton as a reactant and removing external protons as products.

'NADH + Q + H → NAD + QH2'

This fixed the yield of ethanol to around 1.85, which was much closer to the experimental evidence.

After fixing the all the above inconsistencies with the model, I performed simulations on the modified genome scale model. The results of the simulations are discussed in the following chapter.

Chapter 3

Results & Discussions

This Chapter includes the simulation results I performed on my modified genome scale model of Zymomonas mobilis ZM4. I have used COBRA v2.0 in Matlab R2010a to perform the simulations. Each section of this chapter deals with simulation results of a different carbon source – glucose, glycerol and xylose/arabinose spanning various possible products from termination at any of the four steps of β-oxidation cycle reversal. The following sub-sections deal with these various products which can be grouped as fatty acids, fatty alcohols and alkanes, varying with the presence of hydroxyl, enoyl or oxo substitution. The simulation results present ***Solution spaces*** of cell growth rate against product formation rate, which graphically presents the relation between the both after the removal of other undesired metabolites such as succinate.

I have analyzed the ***solution space*** trends and follow up tests which verify the reason behind the observed trend. While some production rates showed

increasing trend and some showed decreasing trend, others showed no relation with increasing carbon chain length. For the cases showing ***solution space*** trend independent of chain length, the flux values show the reason being the presence of an optional pathway for pyruvate conversion. It can either produce NADH and CO_2 or just Formic acid (HCOOH). The variation in carbon chain length causes variation in redox equivalents which is taken care by varying the flux distribution between the above two pathways. The redox analysis makes a general comparison between the reducing equivalents and proton requirements for specific product formation and transport. The findings help in understanding the flux flow across the model. The increasing trend in solution space has also been explained by the redox analysis. The reason behind the decreasing trend in solution space was due to the fact that there were protons involved in transport of the product. The productivity and yield analysis draw out the comparison between various carbon sources names glucose, glycerol and xylose/arabinose. As mentioned before all the analysis is under anaerobic or no oxygen consumption conditions.

The section below shows comparison of the results from modified model with the base model as well as experimental results to validate the model. This is followed by a flux map analysis to validate the model by identifying presence of important pathways such as Entner Doudoroff pathway. After the validation a detailed analysis of the different groups of solution spaces is presented along with various tests to identify the reasons for the observed behavior or trend.

3.1. Model validation

I have compared the simulation results from modified model with experimental values to validate the model. The results have been present in Table 1 which shows the comparison of ethanol yield across various conditions along with experimental results. The values from modified model indicate similarity with experimental evidence.

Table 1 - comparison of ethanol yield for model ZmoMBEL601 before and after modifications along with experimental results to establish model validity.

	ZmoMBEL601		Modified ZmoMBEL601		Experimental results	
	As reported by Lee et al.		After curation			
	Biomass (h^{-1})	Ethanol yield (mmol/mmol)	Biomass (h^{-1})	Ethanol yield (mmol/mmol)	Biomass (h^{-1})	Ethanol yield (mmol/mmol)
Wild type strain	0.92	0	0.28	1.85	0.5	1.9

After comparing the yield values, analyzing the flux map also validates the model by sowing the presence of Entner-Doudoroff pathway while producing the desired product. The Entner-Doudoroff pathway has been reported to be active in Zymomonas; hence its presence validates the model. The Figure 3.1 shows the flux map analysis of the modified model ZmoMBEL601 while producing heptadecane, one of the desired products. The figure shows the presence of Entner-Doudoroff pathway after which the carbon flux enters the reversed β-oxidation cycle and finally into the product heptadecane.

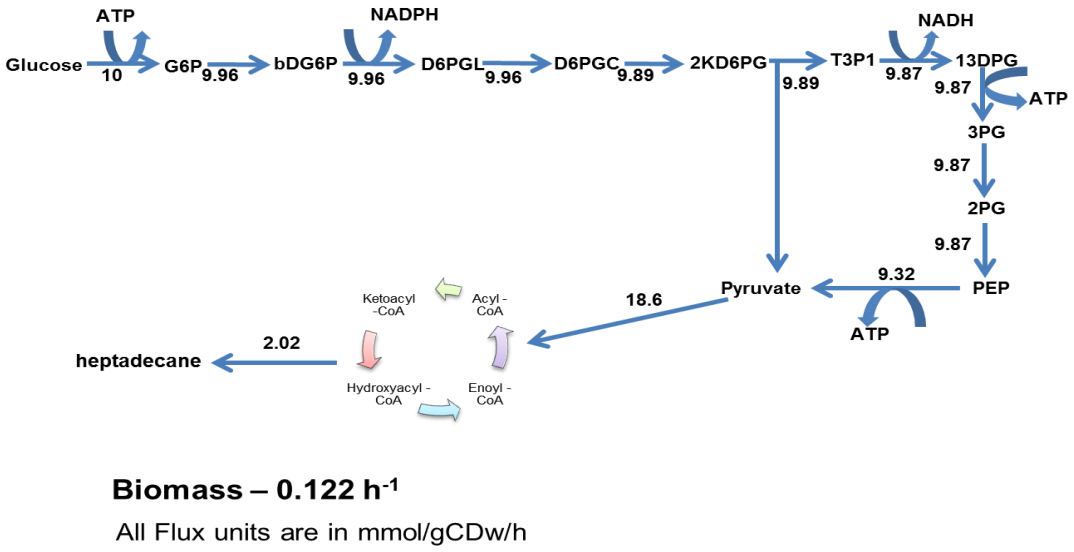


Figure 3.1 – Flux map of modified model ZmoMBEL601 shwoing the presence of Entner-Doudoroff pathway. The carbon flux enters the reverse β-oxidation cycle after pyruvate and terminates to produce heptadecane. The presence of the pathways and products validate the model.

After validating the model I have performed simulations on the model to produce desired products to obtain ***solution spaces***.

3.2. Solution space

The results of the simulations performed on the model ZmoMBEL601 are obtained in the form of ***solution spaces***. These are enclosed figures which show the no growth case and the optimum growth case. They are obtained by adding up all possible flux values for the desired product while varying the growth rate from the minimum value or no growth case to the maximum value i.e. optimum case. The Figure 3.2 shows an example case of producing butanol using glucose as carbon

source. It was obtained after removing other competing metabolites. It is noteworthy that Figure 3.2 shows a coupling of growth rate with butanol production rate; thus, with increasing biomass more desired product in this case butanol is produced.

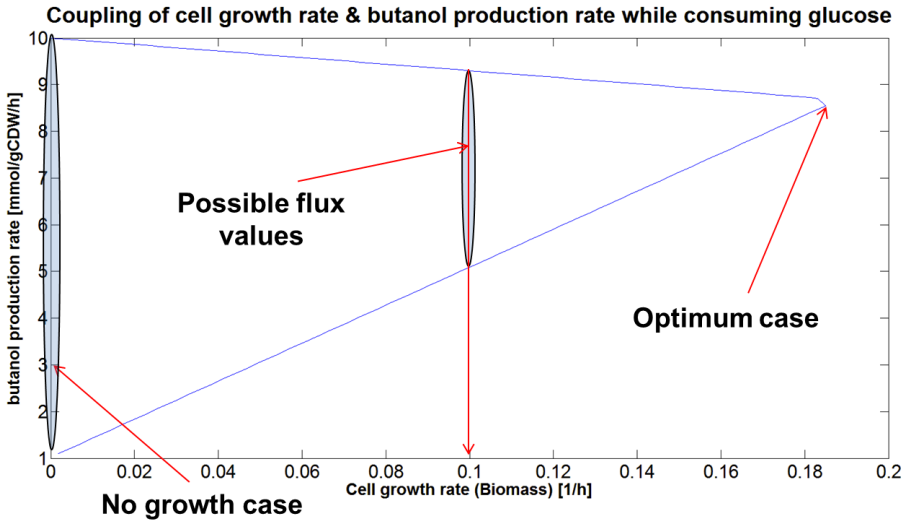


Figure 3.2 – Sample *solution space* showing the optimum case and the no growth case. It is obtained by adding up all possible flux values while varying the growth rate from the optimum case to no growth case.

Analyzing the ***solution spaces*** of all the products showed that they could all be categorized into three different groups. The groups are based on trends in ***solution spaces*** which varied with products and carbon chain length. While some production rates showed increasing trend and some showed decreasing trend, others showed no relation with increasing carbon chain length. The following sections explain the various observed trends and the cause behind the observation.

3.2.1. Solution space case 1: Positive co-relation

Solution spaces of certain group of products showed positive co-relation, i.e. an increasing growth rate trend in solution space was observed with increasing carbon chain length. This was observed while producing all acids, alkanes and alcohols except n-alcohols while using glycerol as a carbon source, under anaerobic conditions with competing metabolites knocked out as can be seen in Figure 3.3 below.

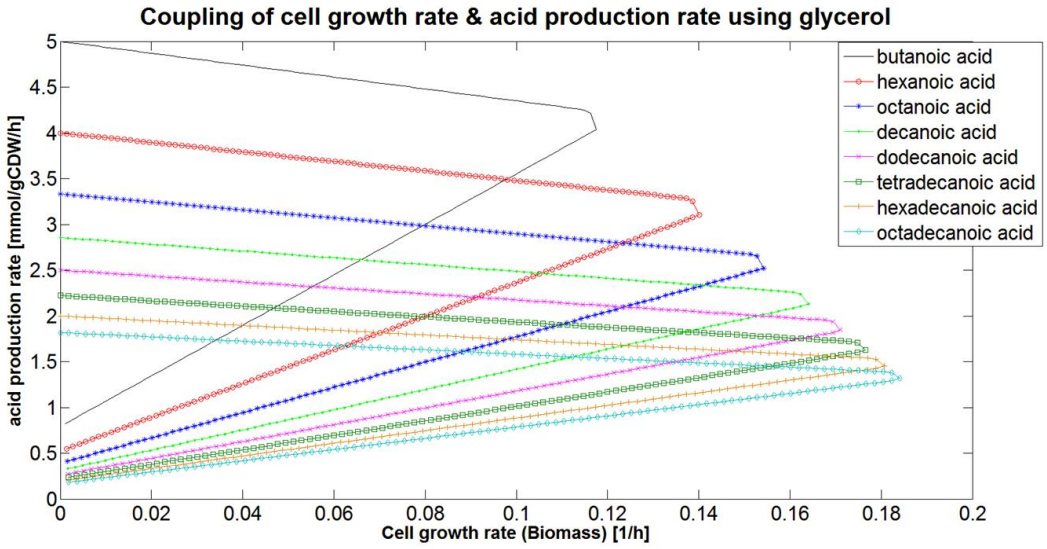
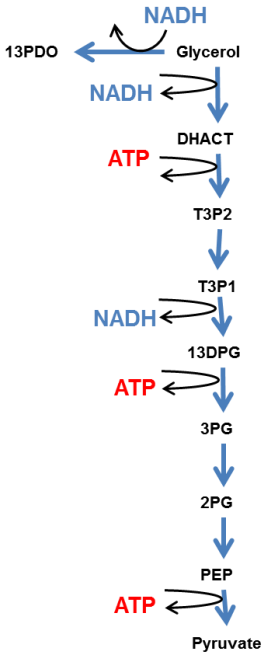
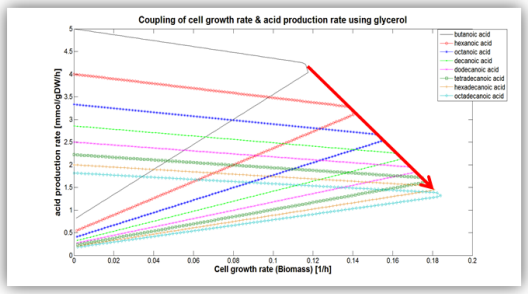


Figure 3.3 - *Solution space* showing an example of positive co-relation with increasing carbon chain length for acid production while using glycerol as carbon source, under anaerobic conditions with competing metabolites knocked out.

This trend of positive co-relation could be explained by following the redox analysis of the products and the carbon metabolism pathway. The following Figure 3.4 shows that 2 redox equivalents are produced up till pyruvate and 2 redox

equivalents are consumed while synthesizing the product. The remaining flux goes into propanediol which doesn't contribute to product or biomass. With increasing carbon chain length the redox consumption increases increasing the carbon flux along pyruvate and reducing it along propanediol; this increases the growth rate with increasing carbon chain length of product.





Products	hydroxy alkane / alkene / acids	
	Consumption of	
Cycles Run	NADH	[H]
1	2	2
2	4	4
3	6	6

Figure 3.4 – Flux map and redox analysis to understand the positive co-relation observed between growth rate and carbon chain length of product. The table shows the redox requirements while producing the desired products which show similar trend.

Thus, redox requirements of the products result in a positive co-relation between growth rate and increasing carbon chain length of product. There was another group of solution spaces which showed negative co-relation as explained in the following section.

3.2.2. Solution space case 2: Negative co-relation

Solution spaces of certain group of products showed negative co-relation, i.e. a decreasing growth rate trend in solution space was observed with increasing carbon chain length. This was observed while producing all alcohols using glucose or xylose and for n-alcohols while using glycerol as a carbon source, under anaerobic conditions with competing metabolites knocked out as can be seen in Figure 3.5 below.

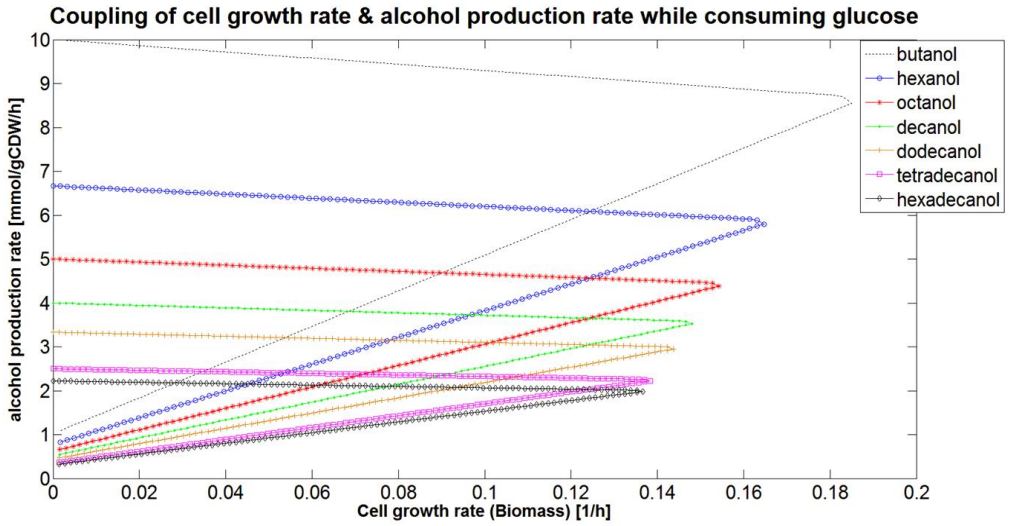
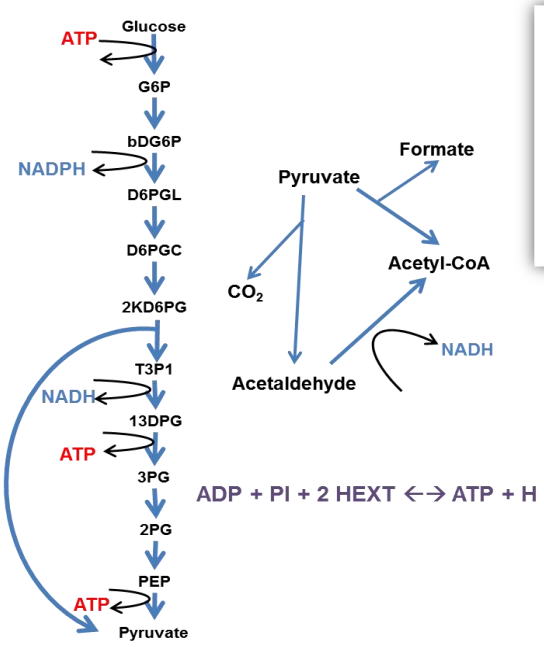
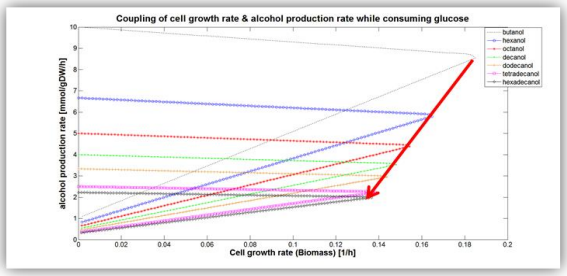


Figure 3.5 - *Solution space* showing an example of negative co-relation with increasing carbon chain length for alcohol production while using glucose as carbon source, under anaerobic conditions with competing metabolites knocked out.

This trend of negative co-relation could not be explained by following the redox analysis of the products and the carbon metabolism pathway. The reason for the trend was proton dependent transport of the product. The following Figure 3.6

shows how the redox requirements are taken care of by diverting the flux at the pyruvate node if there is additional redox requirement. There is however an addition proton required in transport of the product. Due to the relative high concentration of products in case of shorter chain lengths owing to the carbon content, there is higher proton requirement. This addition proton demand drives the proton pump resulting in higher ATP generation and hence higher growth rate for shorter chain length products.





Products	alcohols	
	Consumption of	
Cycles Run	NADH	[H]
1	4	5
2	6	7
3	8	9

Figure 3.6 – Flux map and redox analysis to understand the positive co-relation observed between growth rate and carbon chain length of product. The table shows the redox requirements while producing the desired products which show similar trend.

This behavior is only due to internal protons and not external protons. It is because there is a free transport of external proton present and it shouldn't be

restricted under normal conditions. The internal protons are however constrained by the reactions. I tried running simulations by removing the internal protons involved in alcohol transport as shown by the following Figure 3.7.

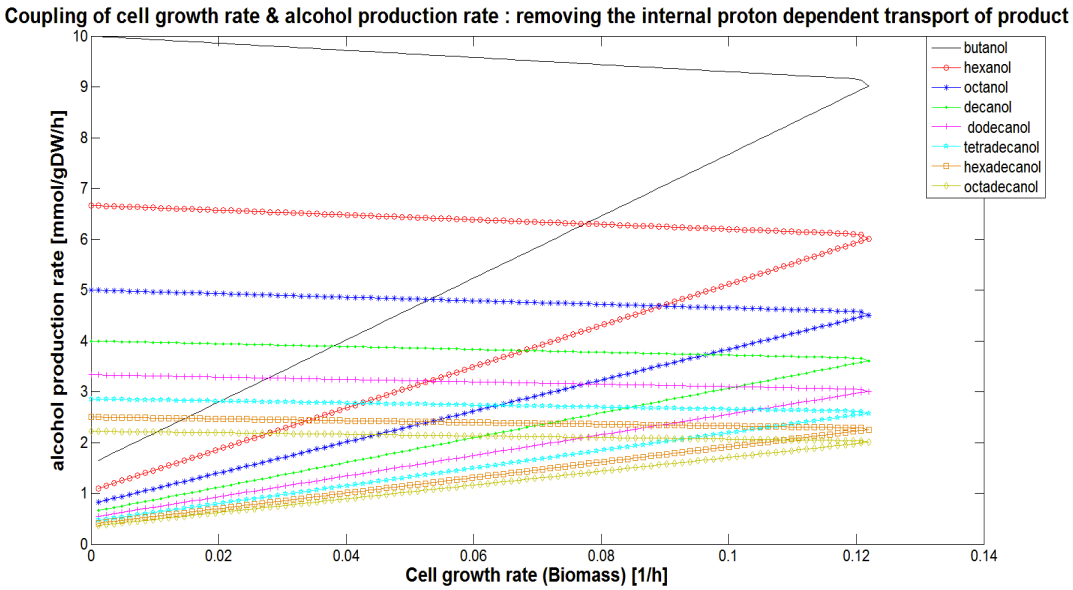


Figure 3.7 - Solution space of cell growth rate Vs. alcohol production rate using glucose as carbon source under anaerobic and proton independent transport conditions.

The above Figure 3.7 can be looked at from the point of view that there is a basic amount of ATP available. Upon export of a larger amount of product the extra internal proton consumption drives more proton production which reduces with increasing chain length as the increasing number of cycles reduces the absolute amount of the final product in turn reducing the gradient produced. This was the case with all alcohol products. Thus, verifying internal protons dependent transport to be responsible for the observed negative co-relation.

Finally, there was a third group of solution spaces which showed no co-relation with increasing carbon chain length as explained in the following section.

3.2.3. Solution space case 3: No co-relation

Solution spaces of the third group of products showed no co-relation with increasing carbon chain length. This was observed while producing all acids and alkanes while using glucose or xylose as a carbon source, under anaerobic conditions with competing metabolites knocked out as can be seen in Figure 3.8 below.

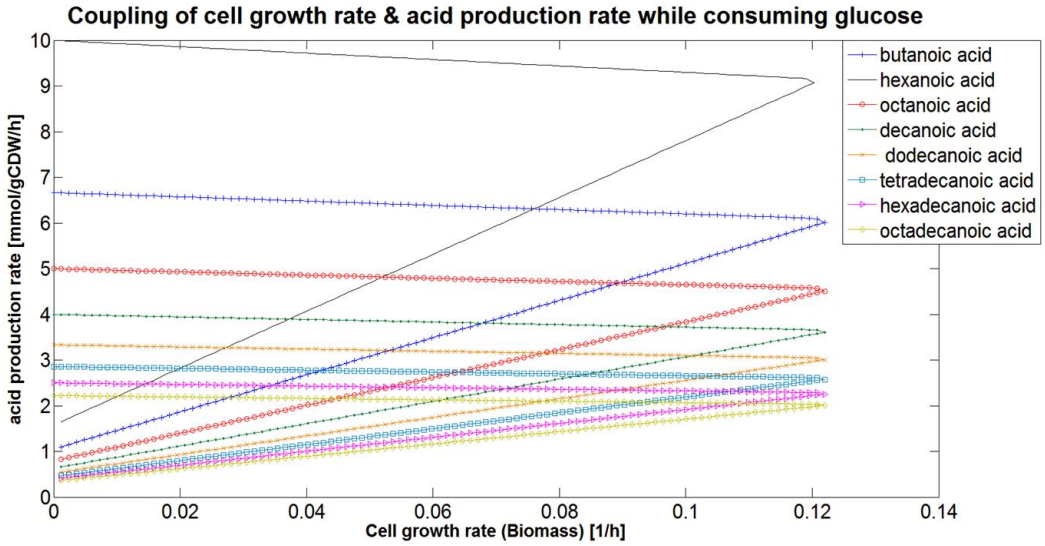
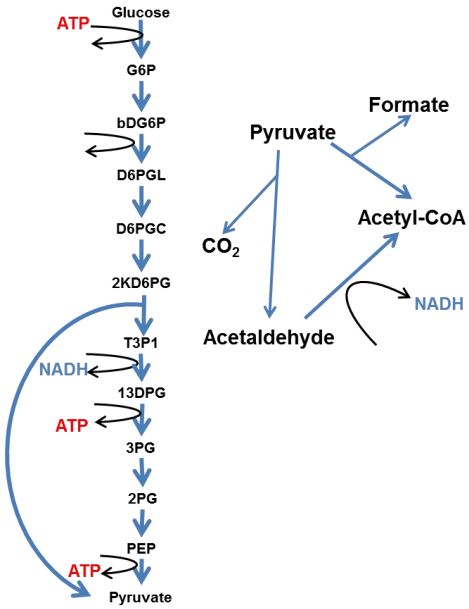
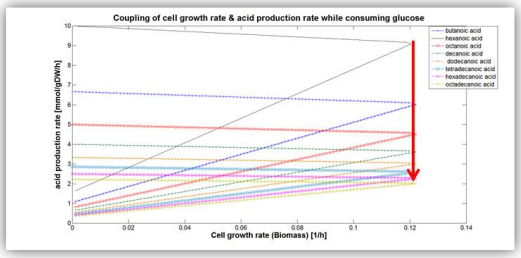


Figure 3.8 - *Solution space* showing an example of no co-relation with increasing carbon chain length for acid production while using glucose as carbon source, under anaerobic conditions with competing metabolites knocked out.

This trend of positive co-relation could be explained by following the redox analysis of the products and the carbon metabolism pathway. The following Figure 3.9 shows that 2 redox equivalents are produced up till pyruvate and 2 redox

equivalents are consumed while synthesizing the product. Even with increasing carbon chain length the addition redox requirements are taken care of. Hence, there is no co-relation observed in this case.





Products	hydroxy alkane / alkene / acids	
	Consumption of	
Cycles Run	NADH	[H]
1	2	2
2	4	4
3	6	6

Figure 3.9 – Flux map and redox analysis to understand no co-relation between growth rate and carbon chain length of certain products. The table shows the redox requirements while producing the desired products which show similar trend.

Thus, redox requirements of the products are being taken care of resulting in no co-relation between growth rate and increasing carbon chain length of product.

3.3. Solution space: Carbon content normalization

After the comparison between different products to observe equivalency, I have also compared various carbon chain lengths. To normalize the comparison between different carbon chains I have compared the production rate of carbon

mmol instead of mmol of the product. I obtained the value by multiplying the number of carbon atoms in the product to its production rate obtaining the production rate in terms of carbon mmol. The results being general to all products I have chosen alcohol production while consuming glucose for the comparison. The following Figure 3.10 shows that the trend in ***solution space*** as observed in the case without the carbon comparison is still maintained.

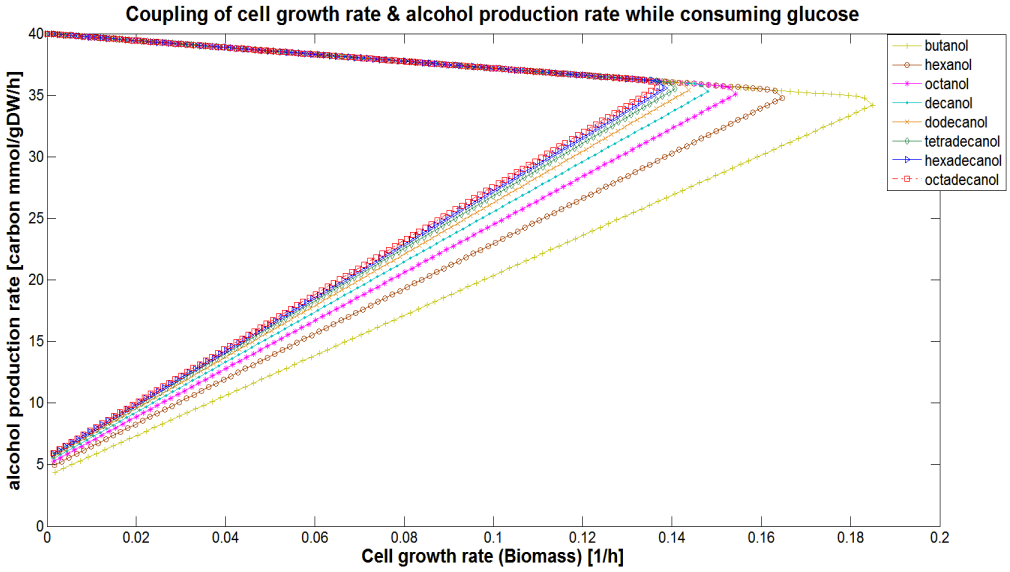


Figure 3.10 - *Solution space* of cell growth rate Vs. alcohol production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. Production rate in terms of carbon mmol to compare for the variation in carbon chain length.

Hence, the previously observed trend in ***solution space*** of various production rates does not change even when analyzed per carbon atom. After this I came across another problem related to solution space associated with formate.

3.4. Solution space: Pyruvate formate-lyase essentiality

Upon following the flux values of various secreted products, I observed that a large amount of formate was being produced as can been seen in the following table.

Table 2 : Flux values of export reactions while producing alcohols using glycerol as carbon source.

Reaction #	Export reaction	Flux value
EX_562	NH3xt <=>	-2.54364
EX_562_1	HEXT <=>	1.148178
EX_564	SLFxt <=>	-0.02939
EX_565	PIxt <=>	-0.0345
EX_566	SUCCxt <=>	0.093229
EX_567	FUMxt <=>	0.00203
EX_574	ACxt <=>	0.022297
EX_576	FORTxt <=>	**13.49708**
EX_587	ACALxt <=>	0.189733
EX_588	GLxt <=>	-20
EX_Biomass	Biomass <=>	0.254173

I removed formate from the model and analyzed the new solution space. Removing formate made the ***solution space*** sub optimal as can be seen in the Figure 3.11.

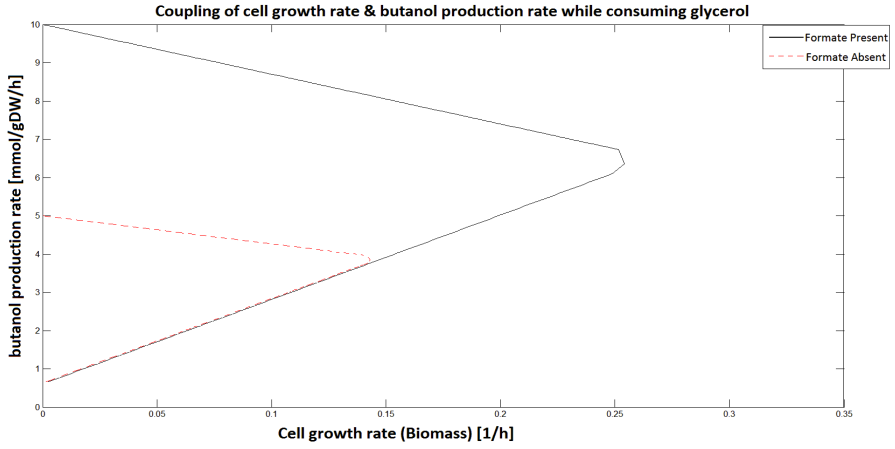


Figure 3.11 – Solution space to analyze the importance of formate presence while using Glycerol as carbon source.

The variation in ***solution space*** in Figure 3.11 is due to the fact that formate production presents an alternate way of pyruvate conversion into Acetyl-CoA with no redox equivalent (NADH) production which can been inferred from the following Figure 3.12.

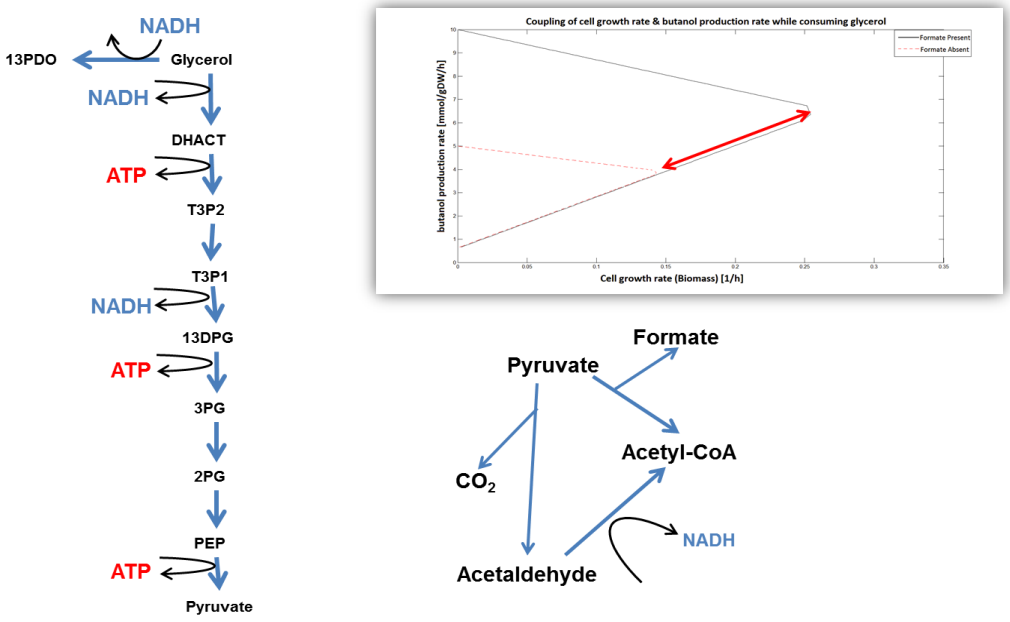


Figure 3.12 - Flux map and redox analysis to understand pyruvate formate lyase essentiality. The presence of the pathway results in less redox equivalent genration as compared to the alternate pathway.

Hence, to obtain higher growth rate and production rate pyruvate formate lyase should not be removed as it produces less redox equivalents.

Having observed redox equivalents affect the system in numerous way I perform a complete redox analysis of all the products produced, independent of the carbon source used.

3.5. Redox analysis

The redox analysis makes a general comparison between various products by comparing their reducing equivalents requirement for formation and proton requirements formation and transport. The redox analysis doesn't differentiate between carbon sources and hence gives a general comparison between products. The following Table 3 highlights the reducing equivalent (NADH) and Internal protons requirement for formation of specific products up to 3 cycles. Products with similar requirements have been grouped together. The results shown are independent of the carbon source used.

Table 3 – Overview of the redox equivalents and internal protons consumption including the product transpost for 1st three runs of the β-oxidation reversal cycle to analyse the obsereved solution space trend with varying products.

	Hydroxy alcohol / Alkenol		Oxo alkane / Hydroxy acids / Enoic acid	
Cycles Run	**NADH consumption**	**[H] consumption**	**NADH consumption**	**[H] consumption**
1	3	4	1	1
2	5	6	3	3
3	7	8	5	5
	Oxo alcohol		**Alcohol**	
Cycles Run	**NADH consumption**	**[H] consumption**	**NADH consumption**	**[H] consumption**
1	2	3	4	5
2	4	5	6	7
3	6	7	8	9
	Hydroxy alkane / Alkene / Acids		**Alkane**	
Cycles Run	**NADH consumption**	**[H] consumption**	**NADH consumption**	**[H] consumption**
1	2	2	3	3
2	4	4	5	5
3	6	6	7	7

Equivalent behavior is observed with optimum growth rate involving those specific products; .i.e. the products grouped under similar redox requirements show similar optimum specific growth rate. As the analysis doesn't depend on the carbon source, I have chosen glycerol for the specific growth rate comparison of products based on their redox requirements. This has been verified by the following Table 4 which shows the optimum growth rates of products with glycerol used as carbon source.

Table 4 – Overview of the optimum case specific growth rates of various products in alignment with the previous redox analysis ;i.e. products with same redox and transport requirements show same growth.

Cycles Run	Optimum case Specific growth rate	Product (s)	Optimum case Specific growth rate	Product (s)	Optimum case Specific growth rate	Product (s)
1	0.186	**Hydroxy alcohol / Alkenol**	0.093	**Oxo alkane / Hydroxy acids / Enoic acids**	0.117	**Hydroxy alkane / Alkene / Acids**
2	0.194		0.117		0.140	
3	0.198		0.133		0.154	
4	0.201		0.145		0.164	
5	0.203		0.154		0.171	
6	0.205		0.161		0.176	
7	0.206		0.166		0.180	
8	0.207		0.171		0.184	
	Optimum case Specific growth rate	**Product (s)**	**Optimum case Specific growth rate**	**Product (s)**	**Optimum case Specific growth rate**	**Product (s)**
1	0.145	**Oxo alcohol**	0.254	**Alcohol**	0.154	**Alkane**
2	0.161		0.242		0.171	
3	0.171		0.235		0.180	
4	0.178		0.232		0.186	
5	0.183		0.229		0.191	
6	0.186		0.227		0.194	
7	0.190		0.226		0.197	
8	0.192		0.225		0.198	

Having analyzed the redox equivalency of the products, I compare the yield and titer values of various products.

3.6. Yield analysis

All the simulations were performed on an equivalent amount (mmol) of carbon basis. Thus, 10 $mmolgCDw^{-1}\ h^{-1}$ was the uptake rate of Glucose, a 6 carbon compound, as compared to 20 $mmolgCDw^{-1}\ h^{-1}$ of Glycerol, a 3 carbon compound, and 12 $mmolgCDw^{-1}\ h^{-1}$ of Xylose/Arabinose, a 5 carbon compound. The following comparison in Table 5 helps us distinguish the various carbon sources based on their yield capacity of the same product.

Table 5 – Product yield comparison of various carbon sources across various products at the end of 8 cycles. All units are mol/mol. The complete list is present at the Appendix B represented by Table 8-12.

Glycerol	Xylose / Arabinose	Glucose	Products
0.07	0.144	0.198	**Hydroxy alcohol / Alkenol**
0.065	0.144	0.198	**Oxo alcohol**
0.062	0.147	0.2	**Oxo alkane / Hydroxy acid / Enoic acid**
0.075	0.144	0.198	**Alcohol**
0.066	0.146	0.2	**Hydroxy alkane / Alkene / Acid**
0.071	0.142	0.2	**Alkane**

It can be seen from the above comparison that the case of glucose shows a much higher yield as compared to the case of glycerol ,which has to divert part of its flux into production of propanediol . Hence, the lower product yields. In case of 5-

carbon compounds such as xylose and arabinose, the yield is better than glycerol because of the non-requirement of additional metabolites. However the ATP generating pathway is less efficient than in case of glucose, as can be inferred from the flux analysis section earlier, hence lesser product yield.

3.7. Titer analysis

Although the yield comparison shows the relation of products per carbon, however it doesn't reveal the total amount of product produced. The following titer comparison shows a more 'practical' comparison between the various carbon sources assuming that the initial biomass concentration was 1gDw/l and after fermenting for 24 hours. The following table shows the amount of product produced per unit volume.

Table 6 – Titer comparison of various carbon sources across various products at the end of 8 cycles after 24 hrs. All units are mol/litre. The complete list is present at the Appendix B represented by Table 8-12.

Glycerol	Xylose / Arabinose	Glucose	Products
0.97	0.54	0.37	**Hydroxy alcohol / Alkenol**
0.68	0.54	0.37	**Oxo alcohol**
0.43	0.43	0.29	**Oxo alkane / Hydroxy acid / Enoic acid**
1.47	0.54	0.37	**Alcohol**
0.59	0.42	0.29	**Hydroxy alkane / Alkene / Acid**
0.84	0.41	0.29	**Alkane**

It can be seen from the above comparison that glycerol still produces the largest amount of products as compared to glucose and xylose/arabinose. It is also noteworthy that though the yield information showed around 2-3 folds difference in cases of various carbon sources, the titer showed the inverse relation. This is due to the fact that the cells show higher growth rate with glycerol as compared to glucose and xylose. Hence, over time the total amount of product produced increased over time. This was true for higher chain lengths. In case of shorter chain lengths xylose/arabinose showed largest titer.

These observations can be explained by following a flux map comparison of the various carbon sources while producing different compounds.

3.8. Flux map analysis

Flux map analysis is performed by obtaining the flux values at various steps by optimizing the cell growth rate. Comparing the optimum case flux maps of the various products across different carbon sources shows the reason behind the difference in yield and titer values. The following Figure 3.13, Figure 3.14 and Figure 3.15 represent the flux maps showing the flux values at various steps in the process of producing heptadecane, octadecanol and octadecanoic acid respectively; from xylose, glycerol and glucose with uptake rates of 12, 20 and 10 $mmol_{gCDw^{-1}}\ h^{-1}$ respectively.

The first noticeable thing in all the flux maps is the presence of the Entner-Doudoroff pathway, which has been mentioned in the introduction chapter as the

major carbon catabolic pathway in Zymomonas. The major flux through the pathway indicates the pathway's importance and validation of the model for supporting this.

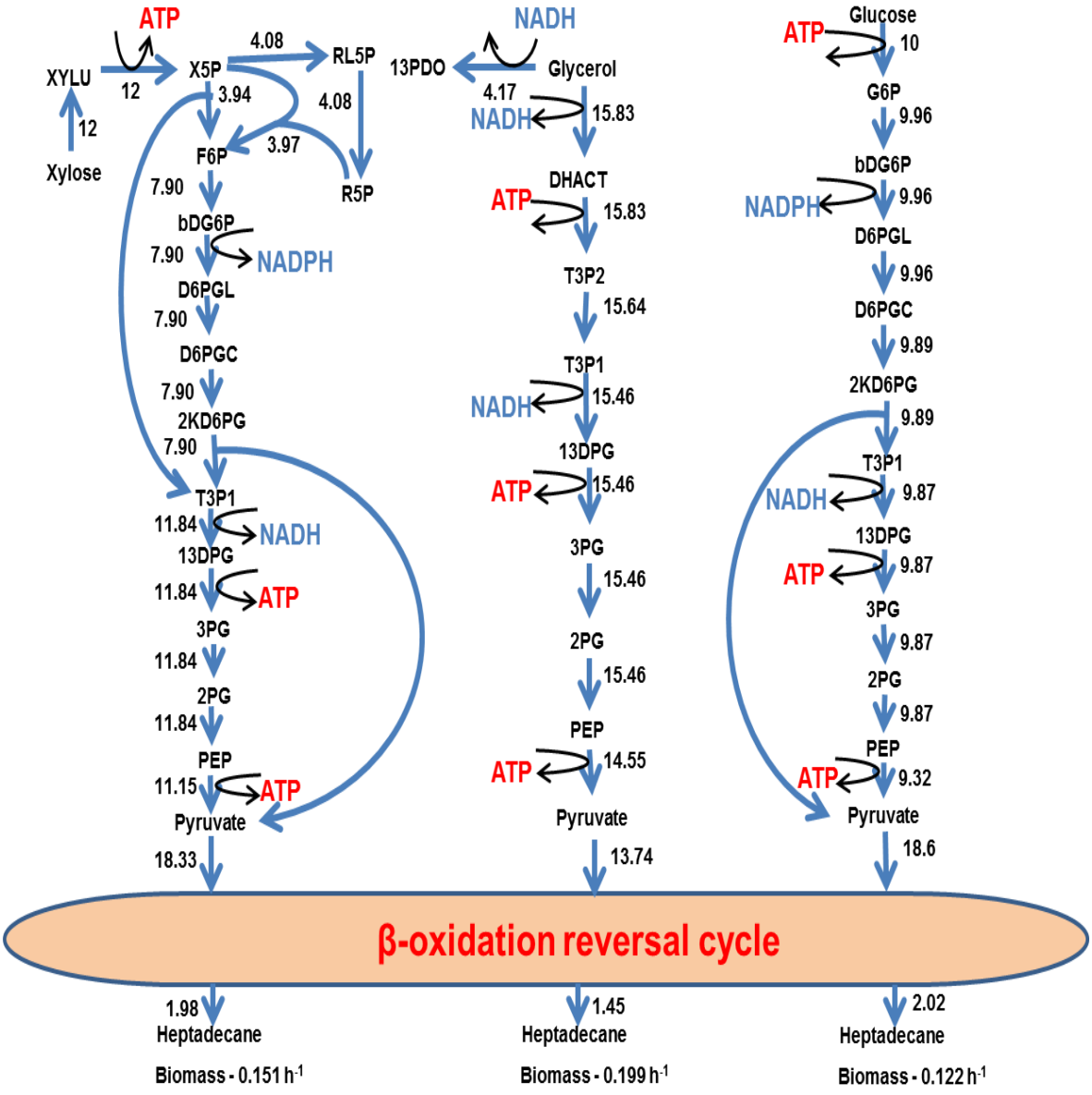


Figure 3.13 - Flux maps of equivalent carbon uptake for different carbon sources, showing flux values at intermediate steps for heptadecane production with uptake rate of 10 mmolgCDw^{-1} h^{-1} for glucose, 12 mmolgCDw^{-1} h^{-1} for xylose and 20 mmolgCDw^{-1} h^{-1} for glycerol. All flux units are in mmolCgDw^{-1} h^{-1}.

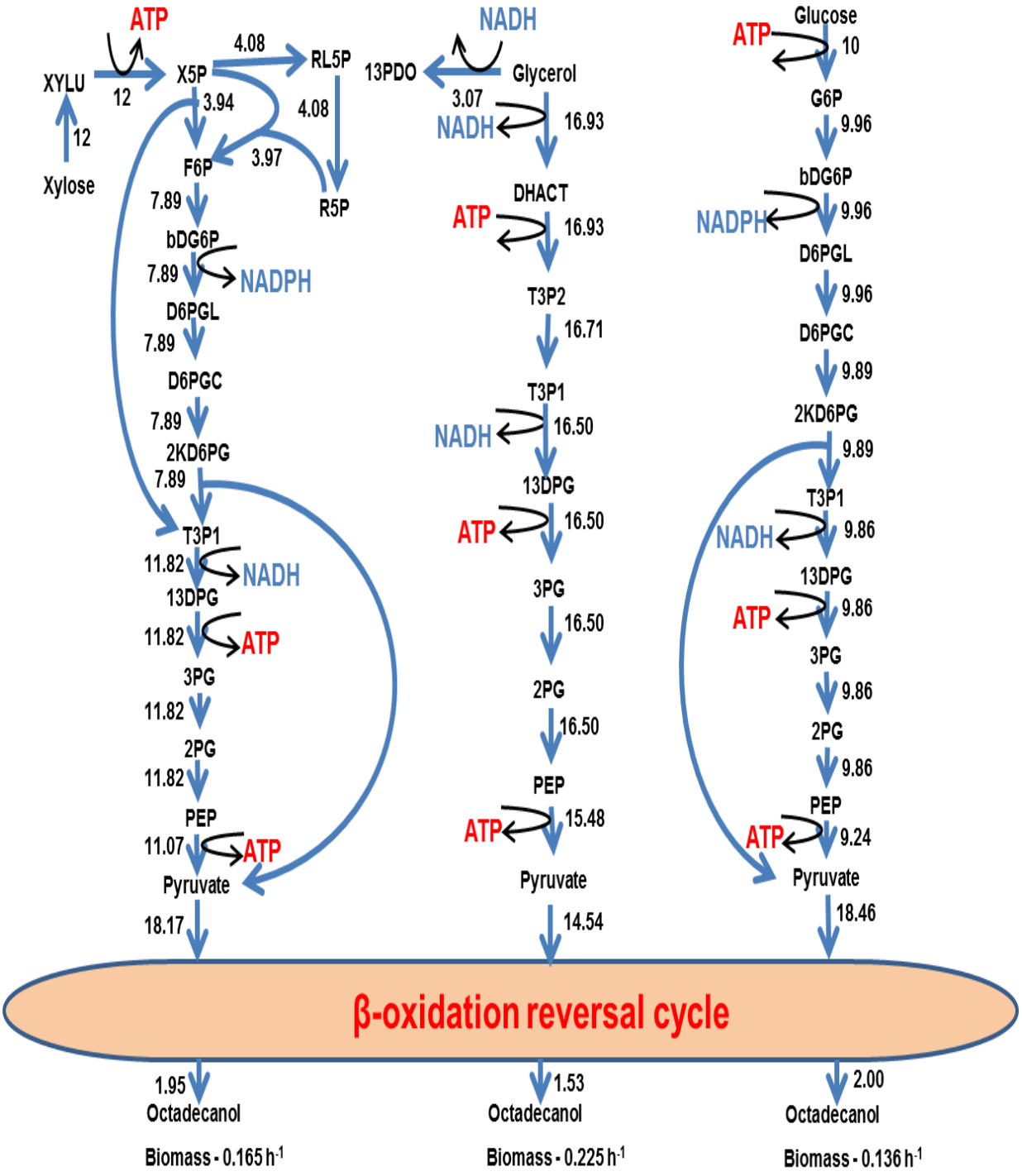


Figure 3.14 - Flux maps of equivalent carbon uptake for different carbon sources, showing flux values at intermediate steps for octadecanol production with uptake rate of 10 mmolgCDw^{-1} h^{-1} for glucose, 12 mmolgCDw^{-1} h^{-1} for xylose and 20 mmolgCDw^{-1} h^{-1} for glycerol. All flux units are in mmolCgDw^{-1} h^{-1}.

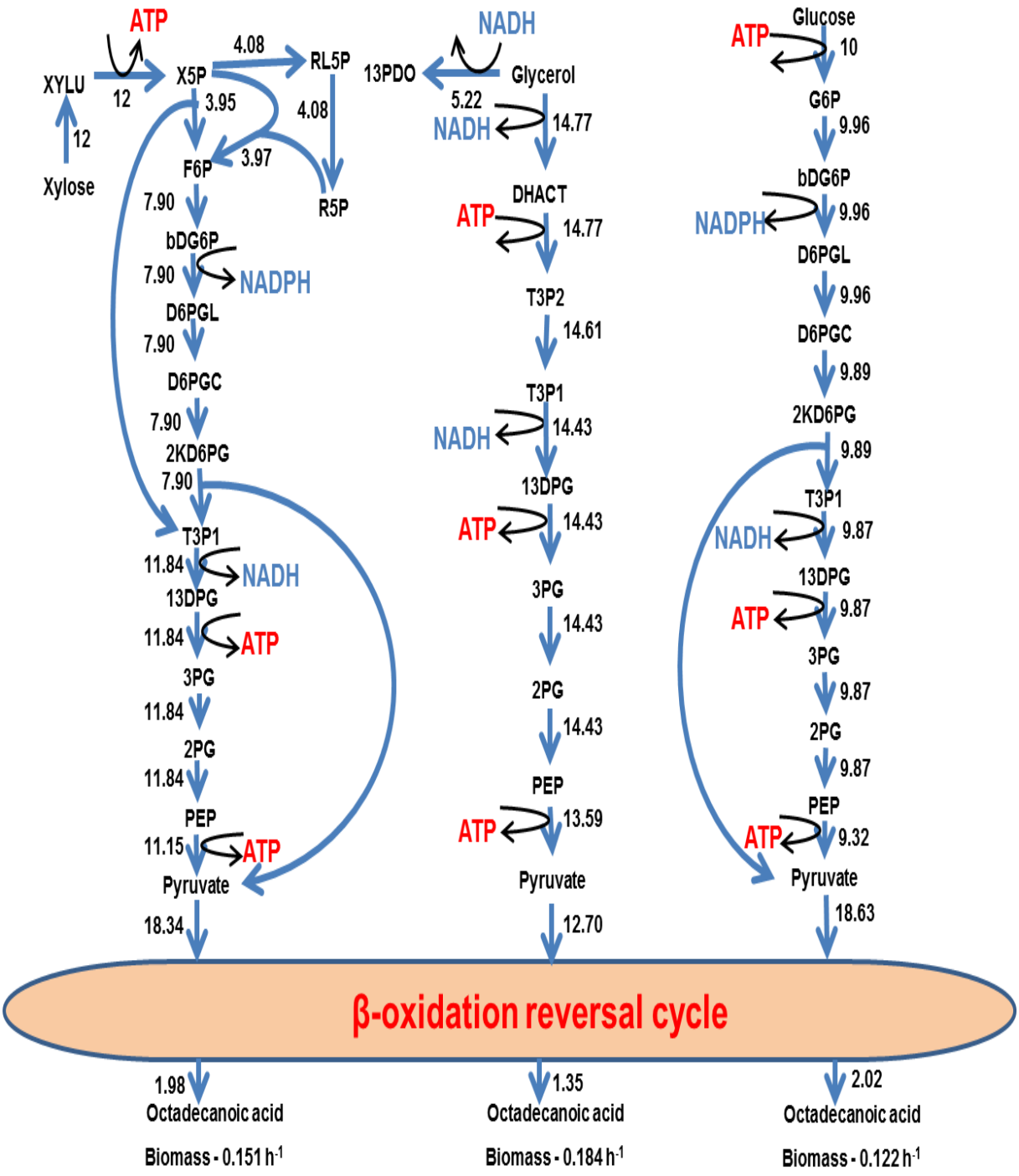


Figure 3.15 - Flux maps of equivalent carbon uptake for different carbon sources, showing flux values at intermediate steps for octadecanoic acid production with uptake rate of 10 mmolgCDw^{-1} h^{-1} for glucose, 12 mmolgCDw^{-1} h^{-1} for xylose and 20 mmolgCDw^{-1} h^{-1} for glycerol. All flux units are in mmolCgDw^{-1} h^{-1}.

Upon following the flux map it can be seen that while the flux is more direct in case of glucose consumption, the case of glycerol has some flux diverted into propanediol. In case of xylose/arabinose there are additional constraints because of the cycles formed in the flux pathways arising due to conversion of 5-carbon compounds into 3 and 6 carbon compounds. Exclusive individual flux maps, with reducing equivalents and ATP, for the case of Heptadecane production using glucose, xylose/arabinose and glycerol are present in appendix C represented by Figure 4.1, Figure 4.2 and Figure 4.3 respectively.

Thus, to summarize from the flux map study, glucose as a carbon source produces less ATP, less biomass and more product flux. Xylose as carbon source produces more ATP than glucose, but less product yield than glucose. In case of xylose the pentose phosphate pathway adds constraints due to formation of loops. Lastly, In case of glycerol as carbon source there is most ATP generated and the higher growth rate or biomass is observed. However, the carbon flux being diverted into propanediol formation results in the least product flux of the three carbon sources. In the end, though glucose is the most effective carbon source in terms of yield but over time the higher growth rate of glycerol dominates resulting in higher product titers for longer carbon chain compounds and xylose for shorter carbon chain length compounds.

After all the above analysis the following chapter concludes and summarizes this study.

Chapter 4

Conclusion

This thesis has presented simulations, showing optimized methods for production of fatty acids, fatty alcohols and alkanes using *Zymomonas mobilis* bacterium by the energy efficient β-oxidation reversal pathway. I have improved a Zymomonas genome scale model developed by Lee et al. and used COBRA (Constraint Based Reconstruction and Analysis), a linear optimization based computational tool in Matlab, to perform FBA (Flux balance analysis) based simulations. I have analyzed the modified model and the results obtained from simulations thereof, gaining insight into the Zymomonas' metabolism, quantitatively presenting efficient product formation and opening way for future improvement.

4.1. Summary

The following represents a summary of the work done in this thesis.

- I have modified the *Zymomonas mobilis* ZM4 genome scale model by Lee et al. to produce fatty acids, fatty alcohols and alkanes.
    - Modifications include addition of upper and lower bound to the flux values of all the reactions in the model.
    - Accounting for the redox balance in the model by removing use of NAD as an external source and by modifying other misrepresented reactions.
    - Addition of internal protons to the whole model by placing them in reactions where they were consumed or produced, solving the problem of excessive cell growth and no product coupling.
    - Adding the β-oxidation reversal cycle to the model and using this pathway to produce the desired products targeted in this study
- I have used COBRA toolbox in Matlab to carry out the simulations using the following specific tools.
    - Flux balance analysis was used to obtain optimum flux distribution across the modified model for model validation and analysis.
    - Flux variability analysis was used to obtain ***solution spaces*** of cell growth rate vs. product formation rate.
- The results, shown by ***solution spaces*** of cell growth rate vs. product formation rate, revealed coupling of the desired products after

removal of unwanted metabolites. The analysis of the various trends of solution spaces varying with carbon chain length and product groups have been explained by the redox analysis.

- Flux map study showed the active presence of the Entner- Doudoroff pathway in the model and the difference in pathways while consuming different carbon sources were revealed.
- Further study using yield and productivity analysis showed glucose as a better carbon source than glycerol or xylose/arabinose.

4.2. Recommendations

Genome scale model of Zymomonas is at the core of this study. The network of biochemical reactions represent the functions performed by the cell at the molecular level which is manifested in physiological forms. Thus, a genome scale model for simulations predicts information much closer to the actual organism. The presence of major pathways such as Entner –Doudoroff pathway ensures the validity of the genome scale model.

There is however limitations to this model due to simplification of certain complex parameters, such as feedback regulations, involved in the actual reactions and not being accounted for by the genome scale model. This model also accounts for a fraction of the actual possible reactions in the organism. Further inclusion of pathways and metabolites would take the model and the prediction further closer to

the actual values. New pathways could be added and simulated to explore the potential of Zymomonas producing new metabolites.

Finally, as this study promises possible efficient production of fatty acids, fatty alcohols and alkanes, the results can be verified by performing experiments under the specified conditions as suggested by the simulation results.

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Appendix A

Table 7 - List of reactions in the modified model

Rxn no.	Enzyme	Reaction
R001	glucose-6-phosphate isomerase	G6P <=> bDG6P
R002	glucose-6-phosphate isomerase	G6P <=> F6P
R003	glucose-6-phosphate isomerase	bDG6P <=> F6P
R004	glucokinase	GLC + ATP <=> G6P + ADP
R005	aldose 1-epimerase	bDGLC <=> GLC
R006	fructose-1,6-bisphosphatase	FDP -> F6P + PI
R007	fructose-bisphosphate aldolase	FDP <=> T3P1 + T3P2
R008	triosephosphate isomerase	T3P1 <=> T3P2
R009	glyceraldehyde-3-phosphate dehydrogenase	PI + T3P1 + NAD <=> NADH + 13DPG
R010	phosphoglycerate kinase	ADP + 13DPG <=> ATP + 3PG
R011	phosphoglycerate mutase	3PG <=> 2PG
R012	enolase	2PG <=> PEP
R013	pyruvate kinase	ADP + PEP -> ATP + PYR
R014	pyruvate dehydrogenase E1 component subunit alpha AND beta	PYR + THPP -> 2HETHPP + CO2
R015	pyruvate dehydrogenase E1 component subunit alpha AND beta	2HETHPP + LIPO -> THPP + ADLIPO
R016	pyruvate dehydrogenase E2 component	ADLIPO + COA -> DLIPO + ACCOA
R017	dihydrolipoamide dehydrogenase	NAD + DLIPO -> NADH + LIPO
R018	pyruvate decarboxylase	PYR -> CO2 + ACAL
R019	alcohol dehydrogenase or S-(hydroxymethyl)glutathione dehydrogenase	NADH + ACAL <=> NAD + ETH
R020	phosphoglyceromutase	G6P <=> G1P
R021	glucokinase	ATP + bDGLC -> bDG6P + ADP
R022	citrate synthase	ACCOA + OA -> COA + CIT
R023	citrate lyase subunit beta	CIT <=> OA + AC
R024	aconitate hydratase 1	CIT <=> ICIT
R025	isocitrate dehydrogenase	ICIT + NADP <=> CO2 + NADPH + AKG
R026	fumarate hydratase, class I	FUM <=> MAL
R027	succinate dehydrogenase	FUM + FADH2 <=> SUCC + FAD
R028	succinyl-CoA synthetase alpha AND beta subunit	ADP + PI + SUCCOA <=> ATP + COA + SUCC

R029	gluconolactonase	GLCNDL -> GLCN
R030	gluconokinase	ATP + GLCN -> ADP + D6PGC
R031	2-dehydro-3-deoxyphosphogluconate aldolase AND 4-hydroxy-2-oxoglutarate aldolase	2KD6PG -> T3P1 + PYR
R032	phosphogluconate dehydratase	D6PGC -> 2KD6PG
R033	6-phosphogluconolactonase	D6PGL -> D6PGC
R034	6-phosphogluconate dehydrogenase	NADP + D6PGC -> CO2 + NADPH + RL5P
R035	glucose-6-phosphate 1-dehydrogenase	bDG6P + NADP <=> NADPH + D6PGL
R036	transketolase	X5P + E4P <=> F6P + T3P1
R037	transketolase	X5P + R5P <=> T3P1 + S7P
R038	ribulose-phosphate 3-epimerase	RL5P <=> X5P
R039	ribose 5-phosphate isomerase B	RL5P <=> R5P
R040	D-Ribose 1,5-phosphomutase	R5P <=> R1P
R041	gluconate 2-dehydrogenase	NADP + GLUC <=> NADPH + 2kGLUC
R042	UDPglucose 6-dehydrogenase	2 NAD + UDPG <=> 2 NADH + UDPGLC
R043	UTP-glucose-1-phosphate uridylyltransferase	G1P + UTP <=> UDPG + PPI
R044	D-sorbitol dehydrogenase (acceptor)	FADH2 + SOB -> FAD + SOT
R045	phosphomannomutase	MAN6P <=> MAN1P
R046	mannose-6-phosphate	MAN6P <=> F6P
R047	fructose-bisphosphate aldolase,class I	F1P <=> T3P1 + T3P2
R048	iron-containing alcohol dehydrogenase	NADP + S6P <=> NADPH + SB1P
R049	beta-galactosidase	LCTS -> GLC + GLAC
R050	beta-fructofuranosidase	RAF -> MELI + FRU
R051	beta-fructofuranosidase	ST -> FRU + G6GG
R052	gluconolactonase	g14l <=> guln
R053	levansucrase	SUC -> GLC + LEVAN
R054	beta-fructofuranosidase	SUC -> GLC + FRU
R055	fructokinase	ATP + FRU <=> F6P + ADP
R056	glucose-1-phosphate adenylyltransferase	ATP + G1P -> PPI + ADPGLC
R057	Glycogen synthase	ADPGLC -> ADP + GLYCOGEN
R058	Glycogen phosphorylase	PI + GLYCOGEN -> G1P
R059	beta-fructofuranosidase	SUC6P -> G6P + FRU
R060	N-acetylglucosamine-6-phosphate deacetylase	AC + GA6P <=> NAGP
R061	bifunctional protein GlmU	UTP + NAG1P <=> PPI + UDPNAG
R062	bifunctional protein GlmU	ACCOA + GA1P -> COA + NAG1P
R063	phosphoglucosamine mutase	GA6P -> GA1P
R064	glucosamine-fructose-6-phosphate aminotransferase(isomerizing)	F6P + GLN <=> GA6P + GLU

R065	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	PEP + UDPNAG -> PI + UDPNAGEP
R066	UDP-N-acetylmuramate dehydrogenase	NADPH + UDPNAGEP -> NADP + UDPNAM
R067	beta-N-acetylhexosaminidase	CTB -> 2 NAGA
R068	UDP-N-acetylmuramate dehydrogenase	NADH + UDPNAGEP -> NAD + UDPNAM
R069	UDP-glucuronate 5'-epimerase	UDPGLC -> UDPIDU
R070	N-acetylmuramic acid-6-phosphate etherase	ACMU6P <=> NAGP + LAC
R071	lactoylglutathione lyase	RGT + MTGYX <=> SLGT
R072	hydroxyacylglutathione	SLGT <=> LAC + RGT
R073	D-lactate dehydrogenase	NADH + PYR <=> NAD + LAC
R074	phosphoenolpyruvate carboxylase	PEP + CO2 -> PI + OA
R075	formate C-acetyltransferase	PYR + COA -> ACCOA + FORT
R076	malate dehydrogenase (oxaloacetate-decarboxylating)	NAD + MAL <=> NADH + PYR + CO2
R077	acetyl-CoA carboxylase carboxyl transferase subunit alpha AND beta or biotin carboxylase AND acetyl-CoA carboxylase, biotin carboxyl carrier protein	ATP + CO2 + ACCOA -> ADP + PI + MALCOA
R078	pyruvate kinase	PEP + NDP -> PYR + NTP
R079	phosphoglycolate phosphatase	2PG -> PPI + GLYCOLATE
R080	gyoxylate AND hydroxypyruvate reductase A	NADP + GLYCOAL <=> NADPH + GLYCOLATE
R081	N-formylglutamate deformylase	FORGLU -> GLU + FORT
R082	2-dehydro-3-deoxyphosphogluconate aldolase AND 4-hydroxy-2-oxoglutarate aldolase	PYR + GLX <=> HYDROXYAKG
R083	formate C-acetyltransferase	COA + OBUT <=> FORT + PPACOA
R084	acetolactate synthase I/II/III large subunit AND acetolactate synthase I/III small subunit	PYR + TPP -> CO2 + HTPP
R085	SUCC-semialdehyde dehydrogenase (NADP+)	NAD + SUCCSAL -> NADH + SUCC
R086	SUCC-semialdehyde dehydrogenase (NADP+)	NADP + SUCCSAL -> NADPH + SUCC
R087	iron-containing alcohol dehydrogenase	NADH + BUTAL <=> NAD + 1BOH
R088	iron-containing alcohol dehydrogenase	NADPH + BUTAL <=> NADP + 1BOH
R089	acetolactate synthase I/II/III large subunit AND acetolactate synthase I/III small subunit	2 PYR -> CO2 + ACLAC
R090	acetolactate decarboxylase	ACLAC -> CO2 + ACTN
R091	succinyl-CoA synthetase alpha AND beta subunit	ATP + COA + ITCN <=> ADP + PI + ITCNCOA
R092	myo-inositol-1(or 4)-monophosphatase	I3P -> PPI + INSTOL
R093	phosphatidylinositol synthase	INSTOL + CDPDG -> CMP + PINSTOL
R094	myo-inositol-1(or 4)-monophosphatase	I4P -> PPI + INSTOL
R095	myo-inositol-1(or 4)-monophosphatase	IP -> PPI + INSTOL

R096	4-phytase AND acid phosphatase	IHKP -> PI + IPKP
R097	Ubiquinol-cytochrome-c reductase	QH2 <=> Q + 4 HEXT
R098	Inorganic pyrophosphatase	PPI -> 2 PI
R099	Polyphosphate kinase	ATP + PPI <=> ADP + PPPI
R100	atpH;ATP synthase delta subunit	ATP <=> ADP + PI + 2 HEXT
R101	ferredoxin-NADP reductase	NADP + RFEDX <=> NADPH + OFRDX
R102	succinate dehydrogenase	FUM + QH2 <=> SUCC + Q
R103		2 FAD + QH2 -> 2 FADH2 + Q + 2 HEXT
R104		NADH + Q + H -> NAD + QH2
R105		NAD + NADPH <=> NADH + NADP
R106		NADH + 0.5 O2 -> NAD
R107	catalase	H2O2 + METHANOL -> FALD
R108	methylenetetrahydrofolate reductase [NAD(P)H]	FADH2 + METTHF <=> FAD + MTHF
R109	S-(hydroxymethyl)glutathione dehydrogenase AND alcohol dehydrogenase	NAD + HMGT -> NADH + FMGT
R110	carbonic anhydrase	H2CO3 <=> CO2
R111	glutamate synthase (NADPH/NADH) small AND large chain	NADPH + AKG + GLN -> NADP + 2 GLU
R112	glutamine synthetase	ATP + GLU + NH3 <=> ADP + PI + GLN
R113	nitrogenase iron protein NifH AND nitrogenase molybdenum iron protein alpha AND beta chain	16 ATP + 8 RFEDX + N2 -> 16 ADP + 16 PI + 8 OFRDX + 2 NH3
R114	glutamate synthase (NADPH/NADH) small AND large chain	NADH + AKG + GLN -> NAD + 2 GLU
R115	nitrilase	RCN -> NH3 + RCO2
R116	sulfate adenylyltransferase subunit 1 AND 2	ATP + SLF -> PPI + APS
R117	nylylsulfate kinase	ATP + APS <=> ADP + PAPS
R118	phosphoadenosine phosphosulfate reductase	PAPS + RTHIO -> PAP + H2SO3 + OTHIO
R119	sulfite reductase (NADPH) hemoprotein alpha AND beta component	3 NADPH + H2SO3 -> 3 NADP + H2S
R120	homoserine O-acetyltransferase	ACCOA + HSER <=> COA + OAHSER
R121	cystathionine beta-lyase	LLCT -> PYR + NH3 + HCYS
R122	phosphoadenylate 3'-nucleotidase	PAP -> PI + AMP
R123		OAHSER + CYS -> AC + LLCT
R124	fabD; malonyl CoA-acyl carrier protein transacylase	MALCOA + ACP <=> COA + MALACP
R125	fabF; 3-oxoacyl-(acyl-carrier protein) synthase	COA + ACACP <=> ACCOA + ACP
R126	Lauric acid (12:0)	10 NADPH + 5 MALACP + ACACP -> 5 CO2 + 10 NADP + 5 ACP + C120ACP

R127	Myristic acid (14:0)	12 NADPH + 6 MALACP + ACACP -> 6 CO2 + 12 NADP + 6 ACP + C140ACP
R128	Myristoleic acid (14:1)	11 NADPH + 6 MALACP + ACACP -> 6 CO2 + 11 NADP + 6 ACP + C141ACP
R129	Palmitic acid (16:0)	14 NADPH + 7 MALACP + ACACP -> 7 CO2 + 14 NADP + 7 ACP + C160ACP
R130	Palmitoleic acid (16:1)	13 NADPH + 7 MALACP + ACACP -> 7 CO2 + 13 NADP + 7 ACP + C161ACP
R131	Stearic acid (18:0)	16 NADPH + 8 MALACP + ACACP -> 8 CO2 + 16 NADP + 8 ACP + C180ACP
R132	Vaccenic acid (18:1)	15 NADPH + 8 MALACP + ACACP -> 8 CO2 + 15 NADP + 8 ACP + C181ACP
R133		C181ACP -> C190ACP
R134	glycerol-3-phosphate dehydrogenase (NAD(P)+)	T3P2 + NADPH <=> NADP + GL3P
R135	glycerol-3-phosphate acyltransferase	0.007 C120ACP + 0.42 C140ACP + 0.007 C141ACP + 0.254 C160ACP + 0.175 C161ACP + 0.013 C180ACP + 0.112 C181ACP + GL3P -> ACP + AGL3P
R136	1-acyl-sn-glycerol-3-phosphate acyltransferase	0.007 C120ACP + 0.42 C140ACP + 0.007 C141ACP + 0.254 C160ACP + 0.175 C161ACP + 0.013 C180ACP + 0.112 C181ACP + AGL3P -> ACP + PA
R137	phosphatidate	PA + CTP <=> PPI + CDPDG
R138	phosphatidylserine synthase	CDPDG + SER -> CMP + PS
R139	CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase	CDPDG + GL3P <=> CMP + PGP
R140	cardiolipin synthase	CDPDG + PG <=> CMP + CL
R141	phosphatidylglycerophosphatase	PGP -> PI + PG
R142	phosphatidylserine	PS -> CO2 + PE
R143	phosphatidylethanolamine N-methyltransferase	PE + SAM -> SAH + PNME
R144		SAM + PNME -> SAH + PNDME
R145		SAM + PNDME -> SAH + PC
R147	glycerophosphoryl diester phosphodiesterase	G3PC -> GL3P + CHO
R148	glycerophosphoryl diester phosphodiesterase	G3PE -> GL3P + ETHA
R149	glycerol-3-phosphate acyltransferase	GL3P + ACYCOA <=> COA + AGL3P
R150	1-acyl-sn-glycerol-3-phosphate acyltransferase	AGL3P + ACYCOA -> COA + PA
R151	aldehyde dehydrogenase	NADPH + DGLYCERATE <=> NADP + GLAL
R152	ceramide glucosyltransferase	UDPG + ACSPG -> UDP +

		GLUCM
R153	beta-galactosidase	bGALbGLCM -> GLAC + GLUCM
R154	ribose-phosphate pyrophosphokinase	ATP + R5P <=> AMP + PRPP
R155	amidophosphoribosyltransferase	GLN + PRPP -> PPI + GLU + PRAM
R156	AMP pyrophosphorylase	PRPP + AD -> PPI + AMP
R157	phosphoribosylamine-glycine ligase	ATP + PRAM + GLY <=> ADP + PI + GAR
R158	phosphoribosylglycinamide formyltransferase 1	GAR + FTHF -> THF + FGAR
R159	phosphoribosylformylglycinamidine synthase	ATP + GLN + FGAR -> ADP + PI + GLU + FGAM
R160	phosphoribosylformylglycinamidine cyclo-ligase	ATP + FGAM -> ADP + PI + AIR
R161	5-(carboxyamino)imidazole ribonucleotide synthase	ATP + CO2 + AIR <=> ADP + PI + NCAIR
R162	5-(carboxyamino)imidazole ribonucleotide mutase	NCAIR <=> CAIR
R163	phosphoribosylaminoimidazolesuccinocarboxamide synthase	ATP + CAIR + ASP <=> ADP + PI + SAICAR
R164	adenyloSUCC lyase	SAICAR <=> FUM + AICAR
R165	phosphoribosylaminoimidazolecarboxamide formyltransferase AND IMP cyclohydrolase	FTHF + AICAR <=> THF + PRFICA
R166	phosphoribosylaminoimidazolecarboxamide formyltransferase AND IMP cyclohydrolase	PRFICA <=> IMP
R167	guanylate kinase	ATP + GMP <=> ADP + GDP
R168	ribonucleoside-diphosphate	RTHIO + GDP -> OTHIO + DGDP
R169	ribonucleoside-triphosphate reductase	RTHIO + GTP -> OTHIO + DGTP
R170	guanylate kinase	ATP + DGMP <=> ADP + DGDP
R171	dGTPase	DGTP -> 3 PI + DG
R172	5'-nucleotidase	DGMP <=> PI + DG
R173	5'-nucleotidase	GMP -> PI + GSN
R174	xanthine phosphoribosyltransferase	PRPP + GN -> PPI + GMP
R175	GMP synthase (glutamine hydrolysing)	ATP + GLN + XMP -> PPI + GLU + AMP + GMP
R176	5'-nucleotidase	XMP -> PI + XTSN
R177	xanthine phosphoribosyltransferase	PRPP + XAN -> PPI + XMP
R178	guanine deaminase	GN -> NH3 + XAN
R179	IMP dehydrogenase	NAD + IMP -> NADH + XMP
R180	5'-nucleotidase	IMP -> PI + INS
R181	adenyloSUCC synthase	ASP + IMP + GTP <=> PI + GDP + ASUC
R182	adenyloSUCC lyase	ASUC <=> FUM + AMP
R183	adenosine deaminase	ADN -> NH3 + INS
R184	5'-nucleotidase	AMP -> PI + ADN
R185	inosine phosphorylase	R1P + AD <=> PI + ADN
R186	adenylate kinase	ATP + AMP <=> 2 ADP

R187	ribonucleoside-triphosphate reductase	ATP + RTHIO -> OTHIO + DATP
R188	ribonucleoside-diphosphate reductase alpha AND beta chain	ADP + RTHIO -> OTHIO + DADP
R189	adenylate kinase	ATP + DAMP <=> ADP + DADP
R190	adenosine deaminase	DA -> NH3 + DIN
R191	5'-nucleotidase	DAMP <=> PI + DA
R194	ADP-ribose pyrophosphatase	ARIB -> R5P + AMP
R195	nucleoside-triphosphate pyrophosphatase	ITP -> PPI + IMP
R196	nucleoside-triphosphate pyrophosphatase	GTP -> PPI + GMP
R197	Guanosine 3',5'-bis(diphosphate) 3'-pyrophosphohydrolase	ppGpp <=> PPI + GDP
R198	exopolyphosphatase	pppGpp <=> PI + ppGpp
R199	pyruvate kinase	PYR + GTP <=> PEP + GDP
R200	pyruvate kinase	PEP + DGDP -> PYR + DGTP
R201	nucleoside-triphosphate pyrophosphatase	DGTP -> PPI + DGMP
R202	GMP synthase (glutamine hydrolysing)	ATP + NH3 + XMP -> PPI + AMP + GMP
R203	nucleoside-triphosphate pyrophosphatase	XTP -> PPI + XMP
R204	pyruvate kinase	PYR + DATP <=> PEP + DADP
R205	dihydroorotase	CAASP <=> DOROA
R206	aspartate carbamoyltransferase catalytic subunit	ASP + CAP -> PI + CAASP
R207	carbamoyl-phosphate synthase large AND small subunit	ATP + CO2 + GLN -> 2 ADP + PI + GLU + CAP
R208	ribonucleoside-triphosphate reductase	UTP + RTHIO -> OTHIO + DUTP
R209	nucleoside diphosphokinase	ATP + DCDP <=> ADP + DCTP
R210	dihydroorotate oxidase	Q + DOROA <=> QH2 + OROA
R211	orotate phosphoribosyltransferase	PRPP + OROA <=> PPI + OMP
R212	orotidine-5'-phosphate decarboxylase	OMP -> CO2 + UMP
R213	cytidylate kinase	ATP + UMP <=> ADP + UDP
R214	nucleoside diphosphokinase	ATP + UDP <=> ADP + UTP
R215	nucleoside diphosphokinase	ATP + CDP <=> ADP + CTP
R216	CTP synthase	ATP + UTP + GLN -> ADP + PI + GLU + CTP
R217	cytidylate kinase	ATP + CMP <=> ADP + CDP
R218	ribonucleoside-triphosphate reductase	RTHIO + CTP -> OTHIO + DCTP
R219	ribonucleoside-triphosphate reductase	RTHIO + CDP -> OTHIO + DCDP
R220	cytidylate kinase	ATP + DCMP <=> ADP + DCDP
R221	nucleoside-triphosphate pyrophosphatase	DUTP -> PPI + DUMP
R222	dTMP kinase	ATP + DUMP <=> ADP + DUDP
R223	ribonucleoside-diphosphate reductase alpha AND beta chain	RTHIO + UDP -> OTHIO + DUDP
R224	thymidylate synthase	METTHF + DUMP -> DHF + DTMP

R225	dTMP kinase	ATP + DTMP <=> ADP + DTDP
R226	nucleoside diphosphokinase	ATP + DTDP <=> ADP + DTTP
R227	5'-nucleotidase	UMP <=> PI + URI
R228	cytidine deaminase	CYTD -> NH3 + URI
R229	5'-nucleotidase	CMP <=> PI + CYTD
R230	5'-nucleotidase	DCMP <=> PI + DC
R231	cytidine deaminase	DC -> NH3 + DU
R232	thymidine kinase	ATP + DU -> ADP + DUMP
R233	5'-nucleotidase	DTMP -> PI + DT
R234	thymidine kinase	ATP + DT -> ADP + DTMP
R235	thioredoxin reductase (NADPH)	NADPH + OTHIO -> NADP + RTHIO
R236	dCTP deaminase	CTP -> UTP + NH3
R237	CTP synthase	ATP + UTP + NH3 -> ADP + PI + CTP
R238	nucleoside-triphosphate pyrophosphatase	UTP -> PPI + UMP
R239	deoxycytidine triphosphate deaminase	DCTP -> NH3 + DUTP
R240	L-aspartate oxidase	O2 + ASP -> OA + H2O2 + NH3
R241	aspartate aminotransferase	AKG + ASP <=> OA + GLU
R242	aspartate 4-decarboxylase	ASP -> CO2 + ALA
R243	asparagine synthetase	ATP + NH3 + ASP -> PPI + AMP + ASN
R244	aspartate racemase	ASP -> DASP
R245	alanine transaminase	PYR + GLU <=> AKG + ALA
R246	glutamic dehydrogenase	NADPH + AKG + NH3 <=> NADP + GLU
R247	glycerate kinase	ADP + 3PG <=> ATP + DGLYCERATE
R248	D-3-phosphoglycerate dehydrogenase	NAD + 3PG -> NADH + PHP
R249	phosphoserine aminotransferase	GLU + PHP -> AKG + 3PSER
R250	phosphoserine phosphatase	3PSER -> PI + SER
R251	L-serine dehydratase	SER <=> PYR + NH3
R252	glycine hydroxymethyltransferase	SER + THF -> METTHF + GLY
R253	5-aminolevulinate synthase	SUCCOA + GLY -> CO2 + COA + ALAV
R254	threonine aldolase	THR <=> ACAL + GLY
R255	threonine aldolase	PHSER -> PI + THR
R256	homoserine kinase type II	ATP + HSER -> ADP + PHSER
R257	homoserine dehydrogenase	NAD + HSER <=> NADH + ASPSA
R258	homoserine dehydrogenase	NADP + HSER <=> NADPH + ASPSA
R259	aspartate-semialdehyde dehydrogenase	NADPH + BASP -> PI + NADP + ASPSA

R260	aspartate kinase	ATP + ASP -> ADP + BASP
R261	gyoxylate AND hydroxypyruvate reductase A	NAD + DGLY <=> NADH + HPYR
R262	gyoxylate AND hydroxypyruvate reductase A	NADP + DGLY <=> NADPH + HPYR
R263	threonine aldolase	ATHR <=> ETH + GLY
R264	serine O-acetyltransferase	ACCOA + SER <=> COA + ASER
R265	cysteine synthase A	H2S + ASER -> AC + CYS
R266	cystathionine beta-lyase	PYR + NH3 + H2S -> CYS
R267	denosylhomocysteinase	SAH <=> HCYS + ADN
R268	5-methyltetrahydrofolatehomocysteine methyltransferase	MTHF + HCYS <=> THF + MET
R269	S-adenosylmethionine synthetase	ATP + MET -> PI + PPI + SAM
R270	adenosylmethionine decarboxylase	SAM <=> CO2 + DSAM
R271	spermidine synthase	DSAM + PTRC -> SPMD + 5MTA
R272	methylthioadenosine nucleosidase	5MTA -> AD + 5MTR
R273	methylthioribose kinase	ATP + 5MTR -> ADP + 5MTRP
R274	aldose-ketose-isomerase	5MTRP <=> 5MTR1P
R275	methylthioribulose 1-phosphate dehydratase	5MTR1P -> DKMPP
R276	acireductone synthase	DKMPP -> FORT + KMB
R277		GLN + KMB -> GLU + MET
R278	L-serine dehydratase	SER -> 2AA
R279	cysteine synthase A	RTHIO + ASER + HO3S2 -> AC + H2SO3 + OTHIO + CYS
R280	cystathionine beta-lyase	CYST -> PYR + NH3 + TCYS
R281	aspartate aminotransferase	AKG + CYSTEATE <=> GLU + 3SPYR
R282	aspartate aminotransferase	AKG + CYS <=> GLU + MPYR
R283	aspartate aminotransferase	AKG + 3SLALA -> GLU + 3SFPYR
R284	aspartate 4-decarboxylase	3SLALA -> ALA + SO2
R285	5-methyltetrahydropteroyltriglutam ate-homocysteine methyltransferase	HCYS + MTGLU -> MET + TGLU
R286	methionine-gamma-lyase	MET -> OBUT + NH3 + MTE
R287	aromatic-amino-acid transaminase	GLU + KMB -> AKG + MET
R288	threonine dehydratase	THR -> OBUT + NH3
R289	acetolactate synthase I/II/III large AND small subunit	OBUT + HTPP -> TPP + ABUT
R290	acetolactate synthase I/II/III large AND small subunit	ABUT <=> HMOPENT
R291	dihydroxy-acid dehydratase	DHMB -> MOBA
R292	acetolactate synthase I/II/III large AND small subunit	PYR + HTPP -> TPP + ACLAC
R293	2-isopropylmalate synthase	ACCOA + MOBA -> COA + IPPMAL
R294	branched-chain amino acid aminotransferase	GLU + MOPENA -> AKG + ILE
R295	branched-chain amino acid aminotransferase	GLU + MOBA -> AKG + VAL

R296	3-isopropylmalate dehydrogenase	NAD + IPPMAL -> NADH + OICAP
R297	branched-chain amino acid aminotransferase	GLU + OICAP <=> AKG + LEU
R299	2-isopropylmalate synthase	NAD + E3MM -> NADH + CO2 + OBUT
R300	3-isopropylmalate dehydrogenase	2MM -> E3MM
R301	3-isopropylmalate AND (R)-2-methylmalate dehydratase large AND small subunit	R2MM -> 2MM
R305	ketol-acid reductoisomerase	ACLAC <=> HMOBA
R306	ketol-acid reductoisomerase	NADPH + HMOPENT <=> NADP + DHMP
R307	ketol-acid reductoisomerase	NADPH + HMOBA <=> NADP + DHMB
R308	dihydroxy-acid dehydratase	DHMP -> MOPENA
R311	3-isopropylmalate AND (R)-2-methylmalate dehydratase large AND small subunit	IPPMAL <=> IPPMALE
R312	dihydrodipicolinate synthase	PYR + ASPSA -> D23PIC
R313	dihydrodipicolinate reductase	NADPH + D23PIC -> NADP + PIP26DX
R314	2,3,4,5-tetrahydropyridine-2-carboxylate Nsuccinyltransferase	SUCCOA + PIP26DX -> COA + NS2A6O
R315	acetylornithine AND N-succinyldiaminopimelate aminotransferase	AKG + NS26DP <=> GLU + NS2A6O
R316	succinyl-diaminopimelate desuccinylase	NS26DP -> SUCC + D26PIM
R317	diaminopimelate epimerase	D26PIM <=> MDAP
R318	diaminopimelate decarboxylase	MDAP -> CO2 + LYS
R319	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase	ATP + MDAP + UDPNAMAG -> ADP + PI + UNAGD
R320	UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase	ATP + UNAGD + AA -> ADP + PI + UNAGDA
R321	homocitrate synthase	ACCOA + AKG -> COA + HCT
R322	argininoSUCC synthase	ATP + ASP + CITR <=> PPI + AMP + ARGSUCC
R323	argininoSUCC lyase	ARGSUCC <=> FUM + ARG
R324	dihydrodipicolinate reductase	NADH + D23PIC -> NAD + PIP26DX
R325	arginase	ARG -> ORN + UREA
R326	acetylornithine AND N-succinyldiaminopimelate aminotransferase	GLU + NAGLUSAL <=> AKG + NAARON
R327	N-acetyl-gamma-glutamyl-phosphate reductase	NADPH + NAGLUYP <=> PI + NADP + NAGLUSAL
R328	acetylglutamate kinase	ATP + NAGLU -> ADP + NAGLUYP
R329	glutamate N-acetyltransferase or amino-acid N-acetyltransferase	ACCOA + GLU -> COA + NAGLU
R330	glutamate 5-kinase	ATP + GLU -> ADP + GLUP
R331	glutamate-5-semialdehyde dehydrogenase	NADPH + GLUP -> PI + NADP + GLUGSAL
R332	ornithine decarboxylase	ORN -> CO2 + PTRC

R333	pyrroline-5-carboxylate	NADP + PRO <=> NADPH + PYCA
R334	arginine N-succinyltransferase	SUCCOA + ARG -> COA + SUCCARG
R335	ornithine cyclodeaminase	ORN <=> NH3 + PRO
R336	pyrroline-5-carboxylate	NAD + PRO <=> NADH + PYCA
R337	succinylglutamic semialdehyde dehydrogenase	NAD + SUCCGLU5SAL -> NADH + SUCCGLU
R338	pyrroline-5-carboxylate	NADH + PYRRHCAR -> NAD + HPRO
R339	pyrroline-5-carboxylate	NADPH + PYRRHCAR -> NADP + HPRO
R340	aspartate aminotransferase	AKG + E4HGLU -> GLU + HYDROXYAKG
R341	agmatine deiminase	AGMAT -> NH3 + NCPTRC
R342	succinylarginine dihydrolase	SUCCARG -> CO2 + 2 NH3 + SUCCORN
R343	N-carbamoylputrescine amidase	NCPTRC -> CO2 + NH3 + PTRC
R344	ornithine transcarbamylase	CAP + ORN <=> PI + CITR
R345		AKG + ORN <=> GLU + PYCA
R346	ornithine transacetylase	GLU + NAARON <=> ORN + NAGLU
R347	ATP phosphoribosyltransferase AND regulatory subunit	ATP + PRPP <=> PPI + PRBATP
R348	phosphoribosyl-ATP pyrophosphohydrolase	PRBATP -> PPI + PRBAMP
R349	phosphoribosyl-AMP cyclohydrolase	PRBAMP -> PRFP
R350	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase	PRFP -> PRLP
R351	cyclase HisF	GLN + PRLP -> GLU + AICAR + DIMGP
R352	imidazoleglycerol-phosphate dehydratase	DIMGP -> IMACP
R353	histidinol-phosphate aminotransferase	GLU + IMACP -> AKG + HISOLP
R354	histidinol-phosphatase	HISOLP -> PI + HISOL
R355	histidinol dehydrogenase	NAD + HISOL -> NADH + HISAL
R356	histidinol dehydrogenase	NAD + HISAL -> NADH + HIS
R357	alcohol dehydrogenase or S-(hydroxymethyl)glutathione dehydrogenase	NAD + DHPEG <=> NADH + DHMA
R358	indolepyruvate decarboxylase	IDPYR -> CO2 + IDACAL
R359	nitrilase	IDACTN -> NH3 + IAC
R360	3-deoxy-7-phosphoheptulonate synthase	PEP + E4P -> PI + 3DDAH7P
R361	3-dehydroquinate synthase	3DDAH7P -> PI + DOT
R362	3-dehydroquinate dehydratase II	DOT <=> DHSK
R363	shikimate 5-dehydrogenase	NADP + SME <=> NADPH + DHSK
R364	shikimate kinase	ATP + SME -> ADP + SME5P
R365	3-phosphoshikimate 1-carboxyvinyltransferase	PEP + SME5P <=> PI + 3PSME
R366	chorismate synthase	3PSME -> PI + CHOR

R367	tryptophan synthase alpha chain	T3P1 + INDOLE <=> IGP
R368	tryptophan synthase beta chain	SER + INDOLE -> TRP
R369	tryptophan synthase alpha AND beta chain	SER + IGP <=> T3P1 + TRP
R370	indole-3-glycerol phosphate synthase	CPAD5P -> CO2 + IGP
R371	phosphoribosylanthranilate isomerase	NPRAN -> CPAD5P
R372	anthranilate phosphoribosyltransferase	PRPP + AN -> PPI + NPRAN
R373	anthranilate synthase component I AND II	NH3 + CHOR -> PYR + AN
R374	anthranilate synthase component I AND II	GLN + CHOR -> PYR + GLU + AN
R375	chorismate mutase	CHOR <=> PHEN
R376	prephenate dehydratase	PHEN <=> CO2 + PHPYR
R377	aromatic-amino-acid transaminase	AKG + PHE <=> GLU + PHPYR
R378	aspartate aminotransferase	AKG + TYR <=> GLU + HPHPYR
R379	aromatic-amino-acid transaminase	ASP + PHEN -> OA + AG
R380	prephenate dehydratase	AG -> CO2 + PHE
R381	cyclohexadienyl dehydrogenase	NAD + AG -> NADH + CO2 + TYR
R382	cyclohexadienyl dehydrogenase	NADP + AG -> CO2 + NADPH + TYR
R383	cystathionine beta-lyase	SLLCT -> PYR + NH3 + SHCYS
R384	adenosylhomocysteinase	ADSHCYS -> ADN + SHCYS
R385	S-adenosylmethionine	ATP + SMET -> PI + PPI + ADSMET
R386	methionine-gamma-lyase	SMET -> OBUT + NH3 + MTSEL
R387	cysteine synthase A	ASER + SELD -> AC + SCYS
R388	sulfate adenylyltransferase subunit 1 AND 2	ATP + SELNT <=> PPI + ADSELNT
R389	adenylylsulfate kinase	ATP + ADSELNT -> ADP + 3PADSELNT
R390	nitrilase	APRCN -> NH3 + ALA
R391	nitrilase	ACYABUT -> GLU + NH3
R392	gamma-glutamyltranspeptidase	GLU + CYAALA -> GLUCYALA
R393	gamma-glutamyltranspeptidase	GLU + APRCN -> GLUAPRCN
R394	L-asparaginase	ASN -> NH3 + ASP
R395	glutamate racemase	GLU <=> DGLU
R396	UDP-N-acetylmuramoylalanine D-glutamate ligase	ATP + DGLU + UDPNAMA -> ADP + PI + UDPNAMAG
R397	UDP-N-acetylmuramate--alanine ligase	ATP + UDPNAM + ALA -> ADP + PI + UDPNAMA
R398	alanine racemase	ALA <=> DALA
R399	D-alanine-D-alanine ligase	ATP + 2 DALA -> ADP + PI + AA
R400	glutamate-cysteine ligase	ATP + GLU + CYS -> ADP + PI + GC
R401	aminopeptidase	GC -> CYS + GLY
R402	glutathione synthase	ATP + GLY + GC -> ADP + PI + RGT

R403	glutathione reductase (NADPH)	NADPH + OGT <=> NADP + 2 RGT
R404	leucyl aminopeptidase	CYSGLY -> CYS + GLY
R405	gamma-glutamyltranspeptidase	RGT -> GLU + CYSGLY
R407	glutathione S-transferase	RGT + RX -> HAL + RSGSH
R408	gamma-glutamyltranspeptidase	RSGSH -> GLU + RSCYSGLY
R409	aminopeptidase N	RSCYSGLY -> GLY + RSCYS
R410	phospho-N-acetylmuramoyl-pentapeptide-transferase	UNAGDA + UDCPP -> PI + UMP + UAGMDA
R411	UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide) pyrophosphoryl-ndecaprenol Nacetylglucosamine transferase	UDPNAG + UAGMDA -> UDP + UAAGMDA
R412		UAAGMDA -> UDCPDP + PEPTIDO
R413	undecaprenyl-diphosphatase	UDCPDP -> PI + UDCPP
R414		DALA + PPEPTIDO -> PEPTIDO + DALAxt
R415	thiamine biosynthesis protein ThiC	AIR -> AHM
R416	thiamine-phosphate pyrophosphorylase	THZP + AHMPP -> PPI + THMP
R417	thiamine-monophosphate kinase	ATP + THMP <=> ADP + TPP
R418	phosphomethylpyrimidine kinase	ATP + AMPMPM -> ADP + AHMPP
R419	thiamine biosynthesis protein ThiC	TYR + C15815 + DX5P -> THZP
R420	thiamine biosynthesis protein ThiC	TYR + C15815 + IMGLY -> THZP
R421	nucleoside-triphosphatase	THMPP -> PI + THMP
R422	GTP cyclohydrolase II or 3,4-dihydroxy 2-butanone 4-phosphate synthase	GTP -> PPI + FORT + D6RP5P
R423	diaminohydroxyphosphoribosylaminopyrimidine deaminase or 5-amino-6-(5-phosphoribosylamino)uracil reductase	D6RP5P -> NH3 + A6RP5P
R424	diaminohydroxyphosphoribosylaminopyrimidine deaminase or 5-amino-6-(5-phosphoribosylamino)uracil reductase	NADPH + A6RP5P -> NADP + A6RP5P2
R425	Hydrolases	A6RP5P2 -> PI + A6RP
R426	GTP cyclohydrolase II or 3,4-dihydroxy 2-butanone 4-phosphate synthase	RL5P -> FORT + DB4P
R427	riboflavin synthase beta chain	A6RP + DB4P -> PI + D8RL
R428	riboflavin synthase alpha chain	2 D8RL -> A6RP + RIBFLV
R429	riboflavin kinase or FMN adenylyltransferase	ATP + RIBFLV -> ADP + FMN
R430	riboflavin kinase or FMN adenylyltransferase	ATP + FMN -> FAD + PPI
R431	4-phytase or acid phosphatase	FMN -> PI + RIBFLV
R432	pyridoxamine 5'-phosphate oxidase	O2 + NH3 + PL <=> H2O2 + PDLA
R433	pyridoxamine 5'-phosphate oxidase	O2 + PDLA5P <=> H2O2 + NH3 + PL5P
R434	pyridoxamine 5'-phosphate oxidase	O2 + PYRDX <=> H2O2 + PL
R435	pyridoxamine 5'-phosphate oxidase	O2 + P5P <=> H2O2 + PL5P

R436	phosphoserine aminotransferase	GLU + OHB <=> AKG + PHT
R437	threonine synthase	PHT -> PI + 4HLT
R438	pyridoxine 5-phosphate synthase	DX5P + AOPP -> PI + P5P
R439	4-hydroxythreonine-4-phosphate dehydrogenase	NAD + PHT -> NADH + AOPOB
R440	NAD+ kinase	ATP + NAD -> ADP + NADP
R441	NAD+ pyrophosphorylase	ATP + NAMN <=> PPI + NAAD
R442	nicotinate-nucleotide adenylyltransferase	ATP + NMN <=> NAD + PPI
R443	NAD+ synthase	ATP + NH3 + NAAD -> NAD + PPI + AMP
R444	nicotinate-nucleotide adenylyltransferase (carboxylating)	PRPP + QA -> CO2 + PPI + NAMN
R445	quinolinate synthase	T3P2 + ISUCC -> PI + QA
R447	5'-nucleotidase	NMN -> PI + RNAM
R448	5'-nucleotidase	NAMN -> PI + NAMNS
R449	L-aspartate oxidase	O2 + ASP -> H2O2 + ISUCC
R450	ketol-acid reductoisomerase	NADPH + ACLAC <=> NADP + MOBA
R451	3-methyl-2-oxobutanoate hydroxymethyltransferase	METTHF + MOBA -> THF + AKP
R452	2-oxopantoate reductase	NADPH + AKP -> NADP + PANT
R453	pantoate--beta-alanine ligase	ATP + PANT + bALA -> PPI + AMP + PNTO
R454	aspartate alpha-decarboxylase	ASP -> CO2 + bALA
R455	type III pantothenate kinase	ATP + PNTO -> ADP + 4PPNTO
R456	phosphopantothenoylcysteine decarboxylase or phosphopantothenate--cysteine ligase	CYS + CTP + 4PPNTO -> PPI + CMP + 4PPNCYS
R457	phosphopantothenoylcysteine decarboxylase or phosphopantothenate--cysteine ligase	4PPNCYS -> CO2 + 4PPNTE
R458	holo-[acyl-carrier protein] synthase	COA -> PAP + ACP
R459	pantetheine-phosphate adenylyltransferase	ATP + 4PPNTE -> PPI + DPCOA
R460	dephospho-CoA kinase	ATP + DPCOA -> ADP + COA
R461	phosphopantothenoylcysteine decarboxylase or phosphopantothenate--cysteine ligase	ATP + CYS + 4PPNTO -> PPI + AMP + 4PPNCYS
R462	type III pantothenate kinase	ATP + PNCYS -> ADP + 4PPNCYS
R463	type III pantothenate kinase	ATP + PTT -> ADP + 4PPNTE
R464	8-amino-7-oxononanoate synthase	ALA + CHCOA <=> CO2 + COA + AONA
R465	adenosylmethionine-8-amino-7-oxononanoate aminotransferase	SAM + AONA <=> SAMOB + DANNA
R466	dethiobiotin synthetase	ATP + CO2 + DANNA <=> ADP + PI + DTB
R467	biotin synthetase	2 SAM + DTB + S -> 2 DA + 2 MET + BT
R468	BirA family transcriptional regulator, biotin operon repressor or biotin-[acetyl-CoA carboxylase] ligase	ATP + BT -> PPI + B5AMP
R469	alkaline phosphatase	AHTD -> 3 PI + DHP

R470	dihydroneopterin aldolase	DHP -> GLAL + AHHMP
R471	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	ATP + AHHMP -> AMP + AHHMD
R472	dihydropteroate synthase	AHHMP + PABA -> DHPT
R473	dihydropteroate synthase	AHHMD + PABA -> PPI + DHPT
R474	dihydrofolate synthase or folylpolyglutamate synthase	ATP + GLU + DHPT -> ADP + PI + DHF
R475	para-aminobenzoate synthetase component I and II	GLN + CHOR -> GLU + ADCHOR
R476	dihydrofolate reductase	NADPH + DHF <=> NADP + THF
R477	aminodeoxychorismate lyase	ADCHOR -> PYR + PABA
R478	GTP cyclohydrolase I	GTP -> FAPNTP
R479	GTP cyclohydrolase I	FAPNTP -> FORT + DAPMNTP
R480	GTP cyclohydrolase I	DAPMNTP -> AHTD
R481	GTP cyclohydrolase I	DATPTHOPAOPM -> AHTD
R482	6-pyruvoyl tetrahydrobiopterin synthase	AHTD -> PPPI + PYTHP
R483	dihydrofolate reductase	NAD + DHF <=> NADH + FL
R484	dihydrofolate reductase	NADP + DHF <=> NADPH + FL
R485	dihydrofolate reductase	2 NADH + FL -> 2 NAD + THF
R486	dihydrofolate reductase	2 NADPH + FL -> 2 NADP + THF
R487	methylenetetrahydrofolate dehydrogenase (NADP+) or methenyltetrahydrofolate cyclohydrolase	FTHF <=> METHF
R488	methylenetetrahydrofolate dehydrogenase (NADP+) or methenyltetrahydrofolate cyclohydrolase	NADPH + METHF <=> NADP + METTHF
R489	formate--tetrahydrofolate ligase	ATP + FORT + THF -> ADP + PI + FTHF
R490	dihydrofolate reductase	NADH + DHF -> NAD + THF
R491	phosphoribosylglycinamide formyltransferase 1	GAR + METHF -> THF + FGAR
R492	5-formyltetrahydrofolate cyclo ligase	ATP + FTHF -> ADP + PI + METHF
R493	hydroxymethylbilane synthase	4 PBG -> 4 NH3 + HMB
R494	uroporphyrinogen-III synthase	HMB -> UPRG
R495	uroporphyrin-III Cmethyltransferase or precorrin-2 dehydrogenase or sirohydrochlorin ferrochelatase	2 SAM + UPRG -> 2 SAH + PC2
R496	uroporphyrinogen decarboxylase	UPRG -> 4 CO2 + CPP
R497	coproporphyrinogen III oxidase	O2 + CPP -> 2 CO2 + PPHG
R498	ferrochelatase	PPIX -> PTH
R499	porphobilinogen synthase	2 ALAV -> PBG
R500	uroporphyrin-III Cmethyltransferase or precorrin-2 dehydrogenase or sirohydrochlorin ferrochelatase	SHCL + Fe -> SHEME
R502	uroporphyrin-III Cmethyltransferase or precorrin-2 dehydrogenase or sirohydrochlorin ferrochelatase	NAD + PC2 -> NADH + SHCL
R503	oxygen-independent coproporphyrinogen III oxidase	2 SAM + CPP -> 2 CO2 + 2 DA + 2 MET + PPHG
R504	4-hydroxybenzoate octaprenyltransferase	4HBZ + OPP -> PPI + O4HBZ

R505	Octaprenyl-hydroxybenzoate decarboxylase	O4HBZ -> CO2 + 2OPPP
R506	ubiquinone biosynthesis protein	NADPH + O2 + 2OPPP -> NADP + 2O6H
R507	3-demethylubiquinone-9 3-methyltransferase	SAM + 2O6H -> SAH + 2OPMP
R508	2-octaprenyl-6-methoxyphenol hydroxylase	NADPH + O2 + 2OPMP -> NADP + 2OPMB
R509	ubiquinone or menaquinone biosynthesis methyltransferase	SAM + 2OPMB -> SAH + 2OPMMB
R510	ubiquinone biosynthesis monooxygenase Coq7	NADPH + O2 + 2OPMMB -> NADP + 2OMHMB
R511	3-demethylubiquinone-9 3-methyltransferase	SAM + 2OMHMB -> QH2 + SAH
R512	4-hydroxybenzoate octaprenyltransferase	PPPP + 4HB -> PPI + H3PPBZ
R513	ubiquinone biosynthesis protein	NADPH + O2 + PPPH -> NADP + PP6HPH
R514	3-demethylubiquinone-9 3-methyltransferase	SAM + PP6HPH -> SAH + PP6MPH
R515	2-octaprenyl-6-methoxyphenol hydroxylase	O2 + PP6MPH -> PP6M14BQ
R516	ubiquinone or menaquinone biosynthesis methyltransferase	SAM + PP6M14BQ -> SAH + PP3M6M14BQ
R517	ubiquinone biosynthesis monooxygenase Coq7	NADPH + O2 + PP3M6M14BQ -> NADP + PP3M5H6M14BQ
R518	3-demethylubiquinone-9 3-methyltransferase	SAM + PP3M5H6M14BQ -> Q + SAH
R519	ubiquinone or menaquinone biosynthesis methyltransferase	SAM + DMQ -> SAH + MQ
R520	ubiquinone or menaquinone biosynthesis methyltransferase	SAM + 2P14NQ -> SAH + PQ
R521	enterobactin isochorismatase	ICHOR -> PYR + DHDHBZ
R522	1-deoxy-D-xylulose-5-phosphate synthase	T3P1 + PYR -> CO2 + DX5P
R523	1-deoxy-D-xylulose-5-phosphate reductoisomerase	NADPH + DX5P -> NADP + MDE4P
R524	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase or 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	CTP + MDE4P -> PPI + CDPMDE
R525	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	ATP + CDPMDE -> ADP + 2PCDPMDE
R526	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase or 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	2PCDPMDE -> CMP + MDECPP
R527		NADH + MDECPP -> NAD + HMB4PP
R528	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	NADH + HMB4PP -> NAD + IPPP
R529	isopentenyl-diphosphate Deltaisomerase	IPPP -> DMPP
R530	geranyltranstransferase	IPPP + DMPP -> PPI + GPP
R531	geranyltranstransferase	IPPP + GPP -> PPI + FPP
R532	squalene synthase	NADPH + 2 FPP -> NADP + 2 PPI + SQL
R533	Isomerases	SQL -> 5 HOPENE
R534	Isomerases	SQL -> HOPANOL

R535		HOPENE -> THBH
R536		UDPNAG + THBH -> THBHGA + THBHET
R537		THBHGA <=> THBHET
R538	(E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase	MDECPP + 2 RFD -> HMB4PP + 2 OFD
R539	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	NADPH + HMB4PP -> NADP + IPPP
R540	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	NADH + HMB4PP -> NAD + DMPP
R541	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	NADPH + HMB4PP -> NADP + DMPP
R542	undecaprenyl pyrophosphate synthetase	FPP + IPP -> PPI + GGPP
R543	undecaprenyl pyrophosphate synthetase	7 IPP + GGPP -> 7 PPI + UDCPP
R544	octaprenyl-diphosphate synthase	IPP + HEPPP -> PPI + OPPPI
R545	myo-inositol-1-phosphate synthase	G6P -> I3P
R546	cyclohexadienyl dehydrogenase	NAD + PHEN <=> NADH + CO2 + HPHPYR
R547	carboxymethylenebutenolidase	DCLCMBO -> DCLOHE
R548	carboxymethylenebutenolidase	CMBO -> 2MAC
R549	4-phytase or acid phosphatase (class A)	NPHP -> PI + NPH
R550	alcohol dehydrogenase or S-(hydroxymethyl)glutathione dehydrogenase	NAD + CLPOL -> NADH + CLAALD
R551	vanillate monooxygenase	NADH + O2 + VAN -> NAD + FALD + DHBZ
R552	esterase or lipase	SULLAC -> 2MAC + HSO3
R553	nitrogenase iron protein NifH AND nitrogenase molybdenumiron protein alpha AND beta chain	ATP + RFD + HC2H -> ADP + PI + OFD + C2H4
R554		NAD + COA + EO -> NADH + ACCOA
R555	carboxymethylenebutenolidase	CLCMBO -> CLMAC
R556	carboxymethylenebutenolidase	PAMN -> ACAC
R557	nitrilase	BZNIT -> NH3 + BZ
R558	3-oxoadipate enol-lactonase	ODHFAC -> OAP
R559	alcohol dehydrogenase or S-(hydroxymethyl)glutathione dehydrogenase	NAD + HMNAPTH -> NADH + NAPTHAH
R560	alcohol dehydrogenase or S-(hydroxymethyl)glutathione dehydrogenase	NAD + NAPTHM -> NADH + NAPTHAH
R561	carboxymethylenebutenolidase	FMUCLAC -> 2MAC + HFA
R562	Ammonia membrane transport	HEXT + NH3xt <=> NH3
R563	Potassium membrane transport	HEXT + Kxt <=> K
R564	Sulphate membrane transport	ATP + SLFxt -> ADP + PI + SLF
R565	Phosphate membrane transport	ATP + PIxt -> ADP + 2 PI
R566	Succinate membrane transport	HEXT + SUCCxt <=> SUCC
R567	Fumarate membrane transport	HEXT + FUMxt <=> FUM

R568	Malate membrane transport	HEXT + MALxt <=> MAL
R569	Sodium membrane transport	NAxt <=> HEXT + NA
R570	Carbon dioxide membrane transport	CO2xt <=> CO2
R571	nucleoside-triphosphatase	ATP -> ADP + PI
R572	Sorbose membrane transport	SOBxt <=> SOB + HEXT
R573	D-Sorbitol membrane transport	SOT <=> HEXT + SOTxt
R574	Acetate membrane transport	AC <=> HEXT + ACxt
R575	Lactate membrane transport	LAC <=> HEXT + LACxt
R576	Formate membrane transport	FORT <=> FORTxt
R577	D-Glucose membrane transport	GLCxt -> GLC
R578	Levan membrane transport	LEVAN <=> LEVANxt
R579	Acetoin membrane transport	ACTN <=> HEXT + ACTNxt
R580		NADxt -> NMNxt + AMPxt
R581	Nicotinamide D-ribonucleotide membrane transport	NMNxt -> NMN
R582		NMNxt -> R5P + NAM
R583	Nicotinate membrane transport	NACxt -> NAC
R584	Ethanol membrane transport	ETH -> HEXT + ETHxt
R585	Oxygen membrane transport	O2xt <=> O2
R586	Sucrose membrane transport	SUCxt -> SUC
R587	Acetaldehyde membrane transport	ACALxt <=> ACAL + HEXT
R588	Glycerol membrane transport	GLxt <=> GL
R589	Dihydroxyacetone membrane transport	DHACTxt <=> DHACT
R590	Pantothenate membrane transport	HEXT + PNTOxt <=> PNTO
R591	Fructose membrane transport	FRUxt <=> FRU
R592	DNA	4.4 ATP + 0.75 DGTP + 0.869 DATP + 0.75 DCTP + 0.869 DTTP -> 4.4 ADP + 4.4 PI + 3.237 PPI + DNA
R593	RNA	0.667 ATP + 0.786 UTP + 0.718 CTP + 0.929 GTP -> 1.24 ADP + 1.24 PI + 3.1 PPI + RNA
R594	Protein	44.92 ATP + 0.308 GLN + 0.307 GLU + 0.037 CYS + 0.383 SER + 1.903 GLY + 0.435 ASP + 2.144 ALA + 0.435 ASN + 0.415 THR + 0.145 MET + 0.672 ILE + 1.057 VAL + 0.672 LEU + 0.447 LYS + 0.317 ARG + 0.391 PRO + 0.146 HIS + 0.093 TRP + 0.019 PHE + 0.122 TYR -> 44.92 ADP + 44.92 PI + PROTEIN
R595	Phospholipid	0.141 PINSTOL + 0.054 PG + 0.118 CL + 0.655 PE + 0.25 PC -> PHOSPHOLIPID
R596	Phospholipid	0.1 C140ACP + 0.1 C160ACP + 0.02 C161ACP + 0.78 C181ACP +

		GL3P -> ACP + AGL3P
R597	Phospholipid	0.1 C140ACP + 0.1 C160ACP + 0.02 C161ACP + 0.78 C181ACP + AGL3P -> ACP + PA
R598	Hopanoids	0.024 HOPENE + 0.07 HOPANOL + 0.11 THBH + 0.692 THBHGA + 0.579 THBHET -> HOPANOIDS
R599	TAGs	0.408 C120ACP + 0.445 C140ACP + 0.222 C141ACP + 0.556 C160ACP + 0.593 C161ACP + 0.111 C180ACP + 1.112 C181ACP + 0.259 C190ACP + 1.235 GL3P -> 1.235 PI + 3.705 ACP + TAG
R600	Smallpool	0.167 NAD + 0.145 COA + 0.149 NADP + 0.141 FAD + 0.01 ACP + 0.249 THF + 1.26 PTRC + 0.765 SPMD + 0.243 FMN -> SMALL_MOLECULES
R601	Biomass	16.45 ATP + 0.025 GLYCOGEN + 0.025 PEPTIDO + 0.027 DNA + 0.195 RNA + 0.605 PROTEIN + 0.053 PHOSPHOLIPID + 0.028 HOPANOIDS + 0.004 TAG + 0.038 SMALL_MOLECULES -> 16.45 ADP + 16.45 PI + Biomass
EX_562	Ammonia transport	NH3xt <=>
EX_562_1	Proton transport	HEXT <=>
EX_563	Potassium transport	Kxt <=>
EX_564	Sulfate transport	SLFxt <=>
EX_565	Phosphate transport	PIxt <=>
EX_566	Succinate transport	SUCCxt <=>
EX_567	Fumarate transport	FUMxt <=>
EX_568	Malate transport	MALxt <=>
EX_569	Sodium transport	NAxt <=>
EX_570	Carbon di-oxide transport	CO2xt <=>
EX_572	Sorbose transport	SOBxt <=>
EX_573	D-Sorbitol transport	SOTxt <=>
EX_574	Acetate transport	ACxt <=>
EX_575	Lactate transport	LACxt <=>
EX_576	Formate transport	FORTxt <=>
EX_577	Glucose transport	GLCxt <=>
EX_578	Levan transport	LEVANxt <=>
EX_579	Acetoin transport	ACTNxt <=>
EX_580	Nicotinamide adenine dinucleotide Transport	NADxt <=>
EX_580_1	Adenosine Monophosphate Transport	AMPxt <=>

EX_581	Nicotinamide D-ribonucleotide Transport	NMNxt <=>
EX_583	Nicotinate Transport	NACxt <=>
EX_584	Ethanol export	ETHxt <=>
EX_585	Oxygen transport	O2xt <=>
EX_586	Sucrose transport	SUCxt <=>
EX_587	Acetaldehyde transport	ACALxt <=>
EX_588	Glycerol transport	GLxt <=>
EX_589	Dihydroxyacetone transport	DHACTxt <=>
EX_590	Pantothenate transport	PNTOxt <=>
EX_591	Fructose transport	FRUxt <=>
EX_Biomass	Biomass export	Biomass <=>
R621	Xylose transport	XYL <=>
R622	Xylose isomerase	XYL <=> XYLU
R623	Xylulokinase	XYLU + ATP <=> X5P + ADP
R624	Arabinose isomerase	ARA <=> RIB
R625	Ribulokinase	RIB + ATP <=> LRL5P + ADP
R626	Ribulose-5-phosphate-4-epimerase	LRL5P <=> X5P
R627	Arabinose transport	ARA <=>
R628	transaldolase	S7P + T3P1 <=> E4P + F6P
R629		O2 + 4 ccfe2 + 4 H -> 4 ccfe3 + 4 HEXT
R630	L-aspartate oxidase	ASP + FUM -> ISUCC + SUCC + H
R631	Acetyl-CoA reductase	ACCOA + NADH + H <=> ACAL + COA + NAD
R632	Acetaldehyde dehydrogenase	ACAL + NAD <=> AC + NADH + H
R633	Glycerol dehydrogenase	GL + NAD -> DHACT + NADH + H
R634	Dihydroxyacetone phosphotransferase	DHACT + ATP -> T3P2 + ADP
R635		GL + NADH + H -> 13PDO + NAD
R636	1,3-Propanediol export	13PDO <=>
BR01	acetoacetyl-CoA transferase	ACCOA + ACAC -> AACOA + AC
BR02	acetyl-CoA C-acetyltransferase	2 ACCOA <=> AACOA + COA
BR03	butanoyl-CoA transferase	ACCOA + BTCOA <=> 3OHCOA + COA
BR04	hexanoyl-CoA C-acetyltransferase	ACCOA + HXCOA <=> 3OOCOA + COA
BR05	octanoyl-CoA transferase	ACCOA + OCCOA <=> 3ODCOA + COA
BR06	decanoyl-CoA C-acetyltransferase	ACCOA + DCACOA <=> 3ODDCOA + COA
BR07	dodecanoyl-CoA transferase	ACCOA + DDCACOA <=> 3OTDCOA + COA
BR08	tetradecanoyl-CoA C-acetyltransferase	ACCOA + TDCOA <=> 3OHDCOA + COA

BR09	hexadecanoyl-CoA transferase	ACCOA + PMTCOA <=> 3OODCOA + COA
BR10	3-hydroxyacetyl-CoA dehydrogenase	AACOA + NADH + H <=> 3HBCOA + NAD
BR11	3-hydroxybutanoyl-CoA dehydrogenase	3OHCOA + NADH + H <=> 3HHCOA + NAD
BR12	3-hydroxyhexanoyl-CoA dehydrogenase	3OOCOA + NADH + H <=> 3HOCOA + NAD
BR13	3-hydroxydecanoyl-CoA dehydrogenase	3ODCOA + NADH + H <=> 3HDCOA + NAD
BR14	3-hydroxydodecanoyl-CoA dehydrogenase	3ODDCOA + NADH + H <=> 3HDDCOA + NAD
BR15	3-hydroxytetradecanoyl-CoA dehydrogenase	3OTDCOA + NADH + H <=> 3HTDCOA + NAD
BR16	3-hydroxyhexadecanoyl-CoA dehydrogenase	3OHDCOA + NADH + H <=> 3HHDCOA + NAD
BR17	3-hydroxyoctadcanoyl-CoA dehydrogenase	3OODCOA + NADH + H <=> 3HODCOA + NAD
BR18	3-hydroxyacetyl-CoA dehydratase	3HBCOA <=> B2COA
BR19	3-hydroxybutanoyl-CoA dehydratase	3HHCOA <=> HX2COA
BR20	3-hydroxyhexanoyl-CoA dehydratase	3HOCOA <=> OC2COA
BR21	3-hydroxyoctanoyl-CoA dehydratase	3HDCOA <=> DC2COA
BR22	3-hydroxydecanoyl-CoA dehydratase	3HDDCOA <=> DD2COA
BR23	3-hydroxydodecanoyl-CoA dehydratase	3HTDCOA <=> TD2COA
BR24	3-hydroxytetradecanoyl-CoA dehydratase	3HHDCOA <=> HDD2COA
BR25	3-hydroxyhexadecanoyl-CoA dehydratase	3HODCOA <=> OD2COA
BR26	butanoyl-CoA dehydrogenase	BTCOA + FAD <=> B2COA + FADH2
BR27	hexanoyl-CoA dehydrogenase	HXCOA + FAD <=> HX2COA + FADH2
BR28	octanoyl-CoA dehydrogenase	OCCOA + FAD <=> OC2COA + FADH2
BR29	decanoyl-CoA dehydrogenase	DCACOA + FAD <=> DC2COA + FADH2
BR30	dodecanoyl-CoA dehydrogenase	DDCACOA + FAD <=> DD2COA + FADH2
BR31	tetradecanoyl-CoA dehydrogenase	TDCOA + FAD <=> TD2COA + FADH2
BR32	hexadecanoyl-CoA dehydrogenase	PMTCOA + FAD <=> HDD2COA + FADH2
BR33	octadecanoyl-CoA dehydrogenase	STCOA + FAD <=> OD2COA + FADH2
BR34	butanoyl-CoA dehydrogenase	BTCOA + NAD <=> B2COA + NADH + H
BR35	hexanoyl-CoA dehydrogenase	HXCOA + NAD <=> HX2COA + NADH + H
BR36	octanoyl-CoA dehydrogenase	OCCOA + NAD <=> OC2COA + NADH + H
BR37	decanoyl-CoA dehydrogenase	DCACOA + NAD <=> DC2COA + NADH + H
BR38	dodecanoyl-CoA dehydrogenase	DDCACOA + NAD <=> DD2COA +

		NADH + H
BR39	tetradecanoyl-CoA dehydrogenase	TDCOA + NAD <=> TD2COA + NADH + H
BR40	hexadecanoyl-CoA dehydrogenase	PMTCOA + NAD <=> HDD2COA + NADH + H
BR41	octadecanoyl-CoA dehydrogenase	STCOA + NAD <=> OD2COA + NADH + H
BR42	butanoic-acid-CoA thioesterase	BTCOA <=> BTA + COA + H
BR43	butanoic acid export	BTA + H -> HEXT
BR44	hexanoic-acid-CoA thioesterase	HXCOA <=> HXA + COA + H
BR45	hexanoic acid export	HXA + H -> HEXT
BR46	octanoic-acid-CoA thioesterase	OCCOA <=> OCTA + COA + H
BR47	octanoic acid export	OCTA + H -> HEXT
BR48	decanoic-acid-CoA thioesterase	DCACOA <=> DCA + COA + H
BR49	decanoic acid export	DCA + H -> HEXT
BR50	dedecanoic-acid-CoA thioesterase	DDCACOA <=> DDCA + COA + H
BR51	dodecanoic acid export	DDCA + H -> HEXT
BR52	tetradecanoic-acid-CoA thioesterase	TDCOA <=> TTDCA + COA + H
BR53	tetradecanoic acid export	TTDCA + H -> HEXT
BR54	hexadecanoic-acid-CoA thioesterase	PMTCOA <=> HDCA + COA + H
BR55	hexadecanoic acid export	HDCA + H -> HEXT
BR56	octadecanoic-acid-CoA thioesterase	STCOA <=> OCDCA + COA + H
BR57	octadecanoic acid export	OCDCA + H -> HEXT
BR58	butanoyl-CoA reductase	BTCOA + NADH + H <=> BUTAL + NAD + COA
BR59	butanol export	1BOH + H -> HEXT
BR60	hexanoyl-CoA reductase	HXCOA + NADH + H <=> HXAL + NAD + COA
BR61	hexanol dehydrogenase	HXAL + NADH + H <=> HXOH + NAD
BR62	hexanol export	HXOH + H -> HEXT
BR63	octanoyl-CoA reductase	OCCOA + NADH + H <=> OCAL + NAD + COA
BR64	octanol dehydrogenase	OCAL + NADH + H <=> OCOH + NAD
BR65	octanol export	OCOH + H -> HEXT
BR66	decanoyl-CoA reductase	DCACOA + NADH + H <=> DCAAL + NAD + COA
BR67	decanol dehydrogenase	DCAAL + NADH + H <=> DCAOH + NAD
BR68	decanol export	DCAOH + H -> HEXT
BR69	dodecanoyl-CoA reductase	DDCACOA + NADH + H <=> DDCAAL + NAD + COA
BR70	dodecanol dehydrogenase	DDCAAL + NADH + H <=> DDCAOH + NAD
BR71	dodecanol export	DDCAOH + H -> HEXT

BR72	tetradecanoyl-CoA reductase	TDCOA + NADH + H <=> TDAL + NAD + COA
BR73	tetradecanol dehydrogenase	TDAL + NADH + H <=> TDOH + NAD
BR74	tetradecanol export	TDOH + H -> HEXT
BR75	hexadecanoyl-CoA reductase	PMTCOA + NADH + H <=> PMTAL + NAD + COA
BR76	hexadecanol dehydrogenase	PMTAL + NADH + H <=> PMTOH + NAD
BR77	hexadecanol export	PMTOH + H -> HEXT
BR78	octanoyl-CoA reductase	STCOA + NADH + H <=> STAL + NAD + COA
BR79	octanol dehydrogenase	STAL + NADH + H <=> STOH + NAD
BR80	octdecanol export	STOH + H -> HEXT
BR81	butaryldehyde decarbonylase	PROP + CO <=> BUTAL
BR82	propane export	PROP ->
BR83	hexanaldehyde decarbonylase	PEN + CO <=> HXAL
BR84	pentane export	PEN ->
BR85	Octanaldehyde decarbonylase	HEP + CO <=> OCAL
BR86	Heptane export	HEP ->
BR87	Decanaldehyde decarbonylase	NON + CO <=> DCAAL
BR88	Nonane export	NON ->
BR89	Dodecanaldehyde decarbonylase	UND + CO <=> DDCAAL
BR90	Undecane export	UND ->
BR91	Tetradecanaldehyde decarbonylase	TRD + CO <=> TDAL
BR92	Tridecane export	TRD ->
BR93	Hexadecanaldehyde decarbonylase	PND + CO <=> PMTAL
BR94	Pentadecane export	PND ->
BR95	Octadecnaldehyde decarbonylase	HPD + CO <=> STAL
BR96	Heptadecane export	HPD ->
BR97	butenoic-acid-CoA thioesterase	B2COA <=> B2A + COA + H
BR98	butenoic-acid-export	B2A + H -> HEXT
BR99	hexenoic-acid-CoA thioesterase	HX2COA <=> HX2A + COA + H
BR100	hexenoic-acid-export	HX2A + H -> HEXT
BR101	octenoic-acid-CoA thioesterase	OC2COA <=> OCT2A + COA + H
BR102	octenoic-acid-export	OCT2A + H -> HEXT
BR103	decenoic-acid-CoA thioesterase	DC2COA <=> DC2A + COA + H
BR104	decenoic-acid-export	DC2A + H -> HEXT
BR105	dodecenoic-acid-CoA thioesterase	DD2COA <=> DD2A + COA + H
BR106	dodecenoic-acid-export	DD2A + H -> HEXT
BR107	tetradecenoic-acid-CoA thioesterase	TD2COA <=> TTD2CA + COA + H

BR108	tetradecenoic-acid-export	TTD2CA + H -> HEXT
BR109	hexadecenoic-acid-CoA thioesterase	HDD2COA <=> HD2CA + COA + H
BR110	hexadecenoic-acid-export	HD2CA + H -> HEXT
BR111	octadecenoic-acid-CoA thioesterase	OD2COA <=> OCD2CA + COA + H
BR112	octadecenoic-acid-export	OCD2CA + H -> HEXT
BR113	butenoyl-CoA reductase	B2COA + NADH + H <=> BUT2AL + NAD + COA
BR114	butenol dehydrogenase	BUT2AL + NADH + H <=> B2OH + NAD
BR115	butenol export	B2OH + H -> HEXT
BR116	hexenoyl-CoA reductase	HX2COA + NADH + H <=> HX2AL + NAD + COA
BR117	hexenol dehydrogenase	HX2AL + NADH + H <=> HX2OH + NAD
BR118	hexenol export	HX2OH + H -> HEXT
BR119	octenoyl-CoA reductase	OC2COA + NADH + H <=> OC2AL + NAD + COA
BR120	octenol dehydrogenase	OC2AL + NADH + H <=> OC2OH + NAD
BR121	octenol export	OC2OH + H -> HEXT
BR122	decenoyl-CoA reductase	DC2COA + NADH + H <=> DC2AL + NAD + COA
BR123	decenol dehydrogenase	DC2AL + NADH + H <=> DC2OH + NAD
BR124	decenol export	DC2OH + H -> HEXT
BR125	dodecenoyl-CoA reductase	DD2COA + NADH + H <=> DD2AL + NAD + COA
BR126	dodecenol dehydrogenase	DD2AL + NADH + H <=> DD2OH + NAD
BR127	dodecenol export	DD2OH + H -> HEXT
BR128	tetradecenoyl-CoA reductase	TD2COA + NADH + H <=> TD2AL + NAD + COA
BR129	tetradecenol dehydrogenase	TD2AL + NADH + H <=> TD2OH + NAD
BR130	tetradecenol export	TD2OH + H -> HEXT
BR131	hexadecenoyl-CoA reductase	HDD2COA + NADH + H <=> HDD2AL + NAD + COA
BR132	hexadecenol dehydrogenase	HDD2AL + NADH + H <=> HDD2OH + NAD
BR133	hexadecenol export	HDD2OH + H -> HEXT
BR134	octadecenoyl-CoA reductase	OD2COA + NADH + H <=> OD2AL + NAD + COA
BR135	octadecenol dehydrogenase	OD2AL + NADH + H <=> OD2OH + NAD
BR136	octadecenol export	OD2OH + H -> HEXT
BR137	butenaldehyde decarbonylase	PROP2 + CO <=> BUT2AL
BR138	propene export	PROP2 ->
BR139	hexenaldehyde decarbonylase	PEN2 + CO <=> HX2AL

BR140	pentene export	PEN2 ->
BR141	Octenaldehyde decarbonylase	HEP2 + CO <=> OC2AL
BR142	Heptene export	HEP2 ->
BR143	Decanaldehyde decarbonylase	NON2 + CO <=> DC2AL
BR144	Nonene export	NON2 ->
BR145	Dodecenaldehyde decarbonylase	UND2 + CO <=> DD2AL
BR146	Undecene export	UND2 ->
BR147	Tetradecenaldehyde decarbonylase	TRD2 + CO <=> TD2AL
BR148	Tridecene export	TRD2 ->
BR149	Hexadecenaldehyde decarbonylase	PND2 + CO <=> HDD2AL
BR150	Pentadecene export	PND2 ->
BR151	Octadecenaldehyde decarbonylase	HPD2 + CO <=> OD2AL
BR152	Heptadecene export	HPD2 ->
BR153	3-hydroxy-butanoic-acid-CoA thioesterase	3HBCOA <=> 3HBA + COA + H
BR154	3-hydroxy-butanoic acid export	3HBA + H -> HEXT
BR155	3-hydroxy-hexanoic-acid-CoA thioesterase	3HHCOA <=> 3HHA + COA + H
BR156	3-hydroxy-hexanoic acid export	3HHA + H -> HEXT
BR157	3-hydroxy-octanoic-acid-CoA thioesterase	3HOCOA <=> 3HOA + COA + H
BR158	3-hydroxy-octanoic acid export	3HOA + H -> HEXT
BR159	3-hydroxy-decanoic-acid-CoA thioesterase	3HDCOA <=> 3HDA + COA + H
BR160	3-hydroxy-decanoic acid export	3HDA + H -> HEXT
BR161	3-hydroxy-dedecanoic-acid-CoA thioesterase	3HDDCOA <=> 3HDDA + COA + H
BR162	3-hydroxy-dodecanoic acid export	3HDDA + H -> HEXT
BR163	3-hydroxy-tetradecanoic-acid-CoA thioesterase	3HTDCOA <=> 3HTDCA + COA + H
BR164	3-hydroxy-tetradecanoic acid export	3HTDCA + H -> HEXT
BR165	3-hydroxy-hexadecanoic-acid-CoA thioesterase	3HHDCOA <=> 3HHCA + COA + H
BR166	3-hydroxy-hexadecanoic acid export	3HHCA + H -> HEXT
BR167	3-hydroxy-octadecanoic-acid-CoA thioesterase	3HODCOA <=> 3HODCA + COA + H
BR168	3-hydroxy-octadecanoic acid export	3HODCA + H -> HEXT
BR169	3-hydroxy-butanoyl-CoA reductase	3HBCOA + NADH + H <=> 3HBAL + NAD + COA
BR170	3-hydroxy-butanoyl dehydrogenase	3HBAL + NADH + H <=> 3HBOH + NAD
BR171	3-hydroxy-butanal export	3HBOH + H -> HEXT
BR172	3-hydroxy-hexanoyl-CoA reductase	3HHCOA + NADH + H <=> 3HHAL + NAD + COA
BR173	3-hydroxy-hexanol dehydrogenase	3HHAL + NADH + H <=> 3HHOH + NAD
BR174	3-hydroxy-hexanol export	3HHOH + H -> HEXT
BR175	3-hydroxy-octanoyl-CoA reductase	3HOCOA + NADH + H <=> 3HOAL + NAD + COA

BR176	3-hydroxy-octanol dehydrogenase	3HOAL + NADH + H <=> 3HOOH + NAD
BR177	3-hydroxy-octanol export	3HOOH + H -> HEXT
BR178	3-hydroxy-decanoyl-CoA reductase	3HDCOA + NADH + H <=> 3HDAL + NAD + COA
BR179	3-hydroxy-decanol dehydrogenase	3HDAL + NADH + H <=> 3HDOH + NAD
BR180	3-hydroxy-decanol export	3HDOH + H -> HEXT
BR181	3-hydroxy-dodecanoyl-CoA reductase	3HDDCOA + NADH + H <=> 3HDDAL + NAD + COA
BR182	3-hydroxy-dodecanol dehydrogenase	3HDDAL + NADH + H <=> 3HDDOH + NAD
BR183	3-hydroxy-dodecanol export	3HDDOH + H -> HEXT
BR184	3-hydroxy-tetradecanoyl-CoA reductase	3HTDCOA + NADH + H <=> 3HTAL + NAD + COA
BR185	3-hydroxy-tetradecanol dehydrogenase	3HTAL + NADH + H <=> 3HTOH + NAD
BR186	3-hydroxy-tetradecanol export	3HTOH + H -> HEXT
BR187	3-hydroxy-hexadecanoyl-CoA reductase	3HHDCOA + NADH + H <=> 3HHDAL + NAD + COA
BR188	3-hydroxy-hexadecanol dehydrogenase	3HDDAL + NADH + H <=> 3HDDOH + NAD
BR189	3-hydroxy-hexadecanol export	3HDDOH + H -> HEXT
BR190	3-hydroxy-octanoyl-CoA reductase	3HODCOA + NADH + H <=> 3HODAL + NAD + COA
BR191	3-hydroxy-octanol dehydrogenase	3HODAL + NADH + H <=> 3HODOH + NAD
BR192	3-hydroxy-octdecanol export	3HODOH + H -> HEXT
BR193	3-hydroxy-butaryldehyde decarbonylase	3HPROP + CO <=> 3HBAL
BR194	3-hydroxy-propane export	3HPROP ->
BR195	3-hydroxy-hexanaldehyde decarbonylase	3HPEN + CO <=> 3HHAL
BR196	3-hydroxy-pentane export	3HPEN ->
BR197	3-hydroxy-Octanaldehyde decarbonylase	3HHEP + CO <=> 3HOAL
BR198	3-hydroxy-Heptane export	3HHEP ->
BR199	3-hydroxy-Decanaldehyde decarbonylase	3HNON + CO <=> 3HDAL
BR200	3-hydroxy-Nonane export	3HNON ->
BR201	3-hydroxy-Dodecanaldehyde decarbonylase	3HUND + CO <=> 3HDDAL
BR202	3-hydroxy-Undecane export	3HUND ->
BR203	3-hydroxy-Tetradecanaldehyde decarbonylase	3HTRD + CO <=> 3HTAL
BR204	3-hydroxy-Tridecane export	3HTRD ->
BR205	3-hydroxy-Hexadecanaldehyde decarbonylase	3HPND + CO <=> 3HHDAL
BR206	3-hydroxy-Pentadecane export	3HPND ->
BR207	3-hydroxy-Octadecnaldehyde decarbonylase	3HHPD + CO <=> 3HODAL
BR208	3-hydroxy-Heptadecane export	3HHPD ->
BR209	Acetoacetyl-CoA reductase	AACOA + NADH + H <=> AAAL + NAD + COA

BR210	Acetoacetyl dehydrogenase	AAAL + NADH + H <=> AAOH + NAD
BR211	Acetoacetyl alcohol export	AAOH + H -> HEXT
BR212	3-oxo-hexanoyl-CoA reductase	3OHCOA + NADH + H <=> 3OHAL + NAD + COA
BR213	3-oxo-hexanol dehydrogenase	3OHAL + NADH + H <=> 3OHOH + NAD
BR214	3-oxo -hexanol export	3OHOH + H -> HEXT
BR215	3-oxo-octanoyl-CoA reductase	3OOCOA + NADH + H <=> 3OOAL + NAD + COA
BR216	3-oxo-octanol dehydrogenase	3OOAL + NADH + H <=> 3OOOH + NAD
BR217	3-oxo-octanol export	3OOOH + H -> HEXT
BR218	3-oxo-decanoyl-CoA reductase	3ODCOA + NADH + H <=> 3ODAL + NAD + COA
BR219	3-oxo-decanol dehydrogenase	3ODAL + NADH + H <=> 3ODOH + NAD
BR220	3-oxo-decanol export	3ODOH + H -> HEXT
BR221	3-oxo-dodecanoyl-CoA reductase	3ODDCOA + NADH + H <=> 3ODDAL + NAD + COA
BR222	3-oxo-dodecanol dehydrogenase	3ODDAL + NADH + H <=> 3ODDOH + NAD
BR223	3-oxo-dodecanol export	3ODDOH + H -> HEXT
BR224	3-oxo-tetradecanoyl-CoA reductase	3OTDCOA + NADH + H <=> 3OTDAL + NAD + COA
BR225	3-oxo-tetradecanol dehydrogenase	3OTDAL + NADH + H <=> 3OTDOH + NAD
BR226	3-oxo-tetradecanol export	3OTDOH + H -> HEXT
BR227	3-oxo-hexadecanoyl-CoA reductase	3OHDCOA + NADH + H <=> 3OHDAL + NAD + COA
BR228	3-oxo-hexadecanol dehydrogenase	3OHDAL + NADH + H <=> 3OHDOH + NAD
BR229	3-oxo-hexadecanol export	3OHDOH + H -> HEXT
BR230	3-oxo-octanoyl-CoA reductase	3OODCOA + NADH + H <=> 3OODAL + NAD + COA
BR231	3-oxo-octanol dehydrogenase	3OODAL + NADH + H <=> 3OODOH + NAD
BR232	3-oxo-octdecanol export	3OODOH + H -> HEXT
BR233	3-oxo-butaryldehyde decarbonylase	3OPROP + CO <=> AAAL
BR234	3-oxo-propane export	3OPROP ->
BR235	3-oxo-hexanaldehyde decarbonylase	3OPEN + CO <=> 3OHAL
BR236	3-oxo-pentane export	3OPEN ->
BR237	3-oxo-Octanaldehyde decarbonylase	3OHEP + CO <=> 3OOAL
BR238	3-oxo-Heptane export	3OHEP ->
BR239	3-oxo-Decanaldehyde decarbonylase	3ONON + CO <=> 3ODAL
BR240	3-oxo-Nonane export	3ONON ->
BR241	3-oxo-Dodecanaldehyde decarbonylase	3OUND + CO <=> 3ODDAL

BR242	3-oxo-Undecane export	3OUND ->
BR243	3-oxo-Tetradecanaldehyde decarbonylase	3OTRD + CO <=> 3OTDAL
BR244	3-oxo-Tridecane export	3OTRD ->
BR245	3-oxo-Hexadecanaldehyde decarbonylase	3OPND + CO <=> 3OHDAL
BR246	3-oxo-Pentadecane export	3OPND ->
BR247	3-oxo-Octadecnaldehyde decarbonylase	3OHPD + CO <=> 3OODAL
BR248	3-oxo-Heptadecane export	3OHPD ->
BR249	Carbon monoxide export	CO ->
R210_1	dihydroorotate oxidase	DOROA + FUM <=> SUCC + OROA

Appendix B

Table 8 – Yield and titer analysis of various carbon sources for different cycles of hydroxy alcohol and alkenol.

Cycles	Glycerol		Xylose / Arabinose		Glucose	
	Yield	Productivity	Yield	Productivity	Yield	Productivity
1	0.240	2.26	0.678	6.28	0.8547	3.87
2	0.177	1.93	0.454	2.88	0.5797	1.80
3	0.141	1.67	0.339	1.77	0.4387	1.12
4	0.117	1.46	0.269	1.25	0.3529	0.81
5	0.100	1.30	0.223	0.96	0.295	0.62
6	0.087	1.17	0.189	0.77	0.2536	0.51
7	0.077	1.06	0.163	0.64	0.2224	0.43
8	0.069	0.97	0.144	0.54	0.198	0.37

Table 9 - Yield and titer analysis of various carbon sources for different cycles of oxo-alcohol.

Cycles	Glycerol		Xylose / Arabinose		Glucose	
	Yield	Productivity	Yield	Productivity	Yield	Productivity
1	0.192	0.84	0.701	6.3	0.862	3.75
2	0.149	0.87	0.454	2.88	0.579	1.80
3	0.123	0.86	0.339	1.77	0.438	1.12
4	0.104	0.83	0.269	1.25	0.352	0.81
5	0.090	0.79	0.223	0.96	0.295	0.62
6	0.080	0.75	0.189	0.77	0.253	0.51
7	0.071	0.71	0.163	0.64	0.143	0.28
8	0.064	0.68	0.144	0.54	0.198	0.37

Table 10 - Yield and titer analysis of various carbon sources for different cycles of oxo alkane, hydroxy acid and enoic acid.

Cycles	Glycerol		Xylose / Arabinose		Glucose	
	Yield	Productivity	Yield	Productivity	Yield	Productivity
1	0.169	0.54	0	0	0	0
2	0.1345	0.62	0.49208	1.39	0.6047	0.85
3	0.112	0.65	0.35283	1.03	0.4508	0.65
4	0.096	0.65	0.27567	0.80	0.3607	0.52
5	0.084	0.64	0.22617	0.66	0.3005	0.44
6	0.07485	0.63	0.19175	0.56	0.2576	0.37
7	0.0675	0.61	0.16642	0.48	0.2254	0.33
8	0.06155	0.59	0.147	0.43	0.2004	0.29

Table 11 - Yield and titer analysis of various carbon sources for different cycles of alcohol.

Cycles	Glycerol		Xylose / Arabinose		Glucose	
	Yield	Productivity	Yield	Productivity	Yield	Productivity
1	0.318	1.29	0.678	6.28	0.854	3.87
2	0.217	1.29	0.454	2.88	0.579	1.80
3	0.165	1.22	0.339	1.77	0.438	1.12
4	0.132	1.13	0.269	1.25	0.352	0.81
5	0.111	1.04	0.223	0.96	0.295	0.62
6	0.095	0.96	0.189	0.77	0.253	0.51
7	0.084	0.90	0.163	0.64	0.222	0.43
8	0.074	0.84	0.144	0.54	0.198	0.37

Table 12 - Yield and titer analysis of various carbon sources for different cycles of hydroxy alkane, alkene and acid.

Cycles	Glycerol		Xylose / Arabinose		Glucose	
	Yield	Productivity	Yield	Productivity	Yield	Productivity
1	0.201	0.54	0.738	2.09	0.907	1.28
2	0.155	0.62	0.464	1.35	0.601	0.87
3	0.126	0.65	0.339	0.99	0.450	0.65
4	0.106	0.65	0.267	0.77	0.360	0.52
5	0.092	0.64	0.220	0.64	0.300	0.44
6	0.081	0.63	0.187	0.55	0.257	0.37
7	0.072	0.61	0.163	0.48	0.225	0.33
8	0.065	0.59	0.144	0.42	0.200	0.29

Table 13 - Yield and titer analysis of various carbon sources for different cycles of alkane.

Cycles	Glycerol		Xylose / Arabinose		Glucose	
	Yield	Productivity	Yield	Productivity	Yield	Productivity
1	0.252	1.29	0.678	1.98	0.901	1.31
2	0.184	1.29	0.441	1.28	0.601	0.87
3	0.145	1.22	0.326	0.95	0.450	0.65
4	0.120	1.13	0.259	0.75	0.360	0.52
5	0.102	1.04	0.215	0.63	0.300	0.44
6	0.088	0.96	0.183	0.53	0.257	0.37
7	0.078	0.90	0.160	0.47	0.225	0.33
8	0.070	0.84	0.142	0.41	0.200	0.29

Appendix C

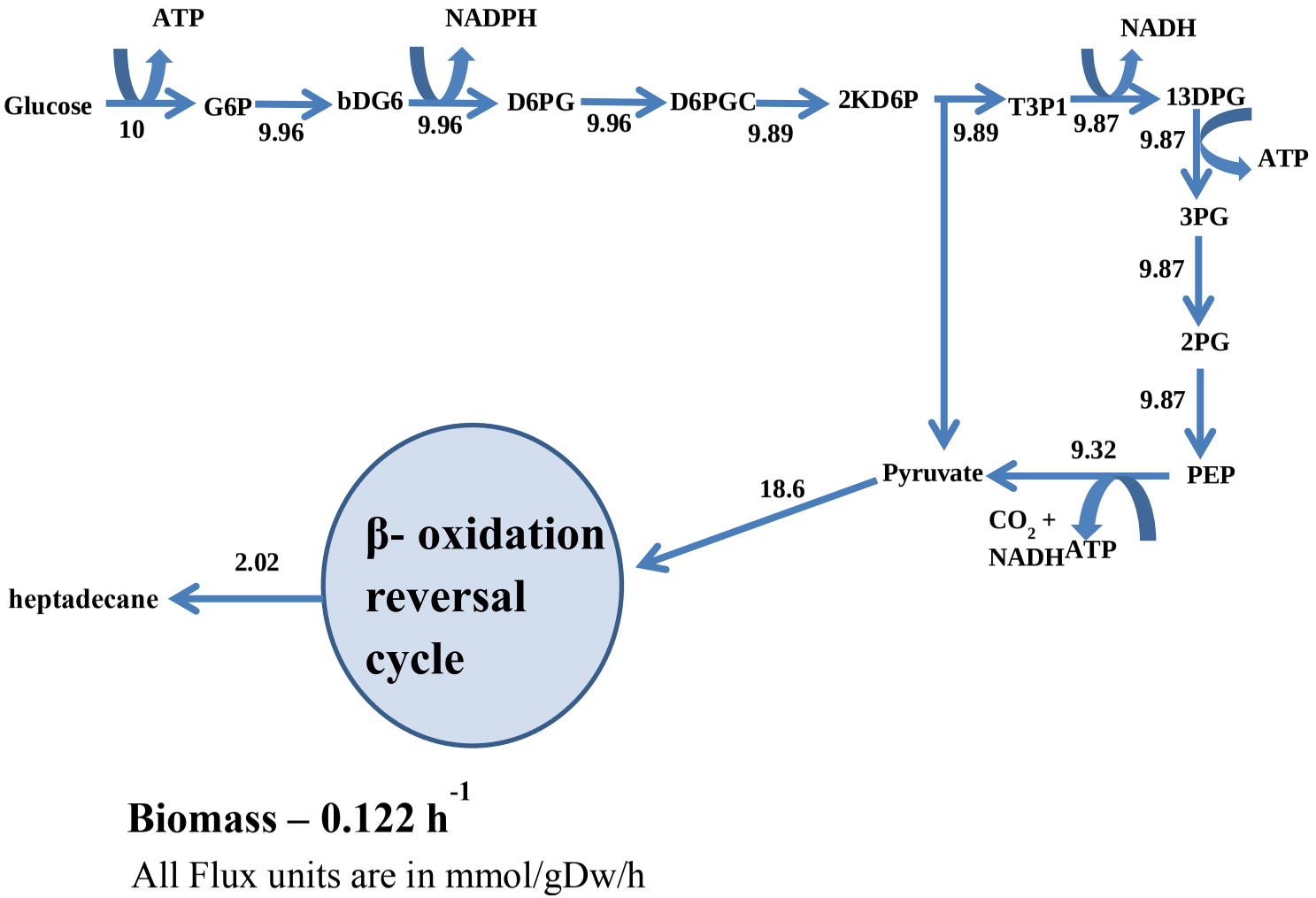
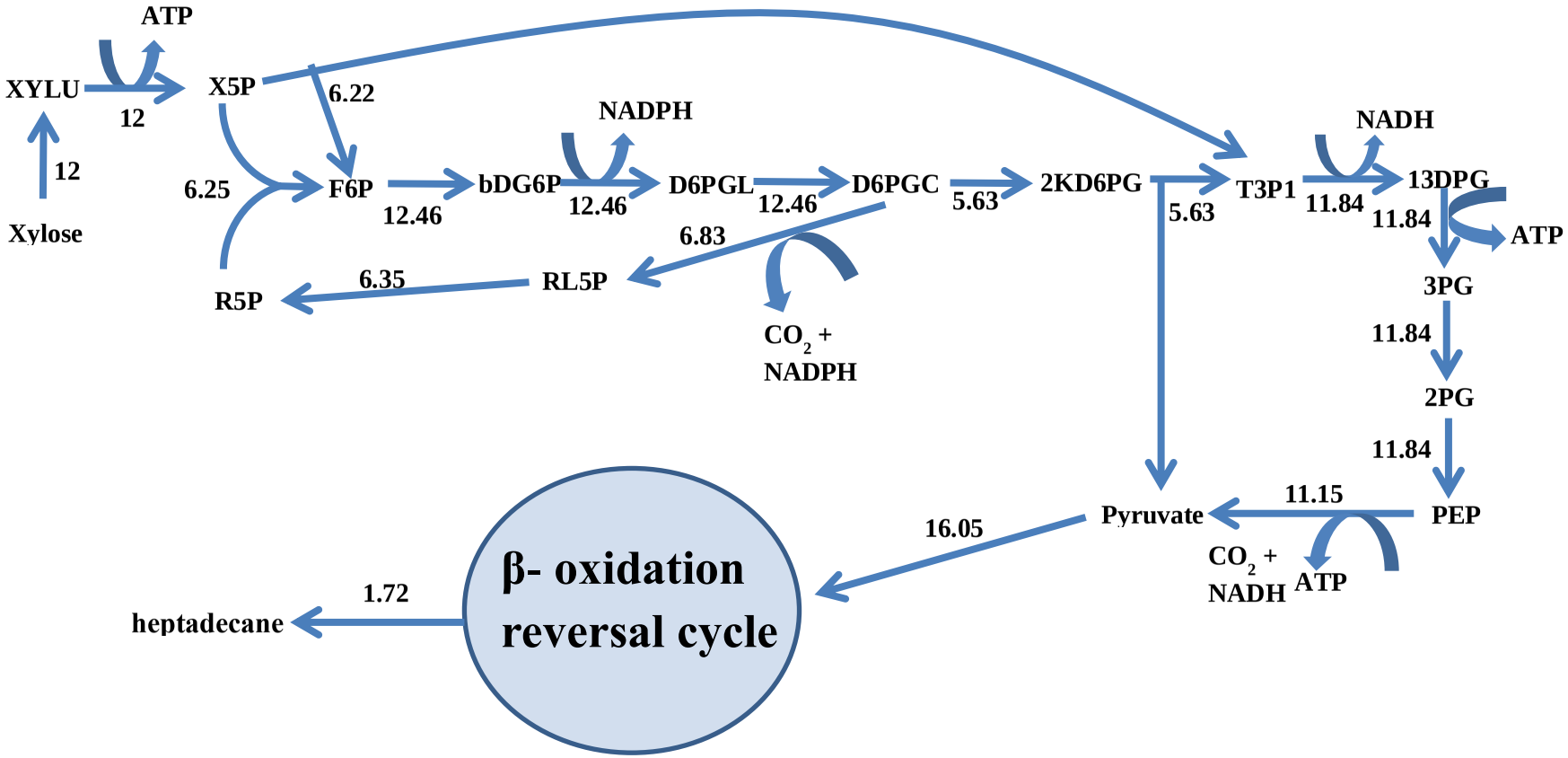


Figure 4.1 – Glucose consuming optimum case flux map while producing heptadecane.



Biomass – 0.151 h^{-1}

All Flux units are in mmol/gDw/h

Figure 4.2 – Xylose consuming optimum case flux map while producing heptadecane.

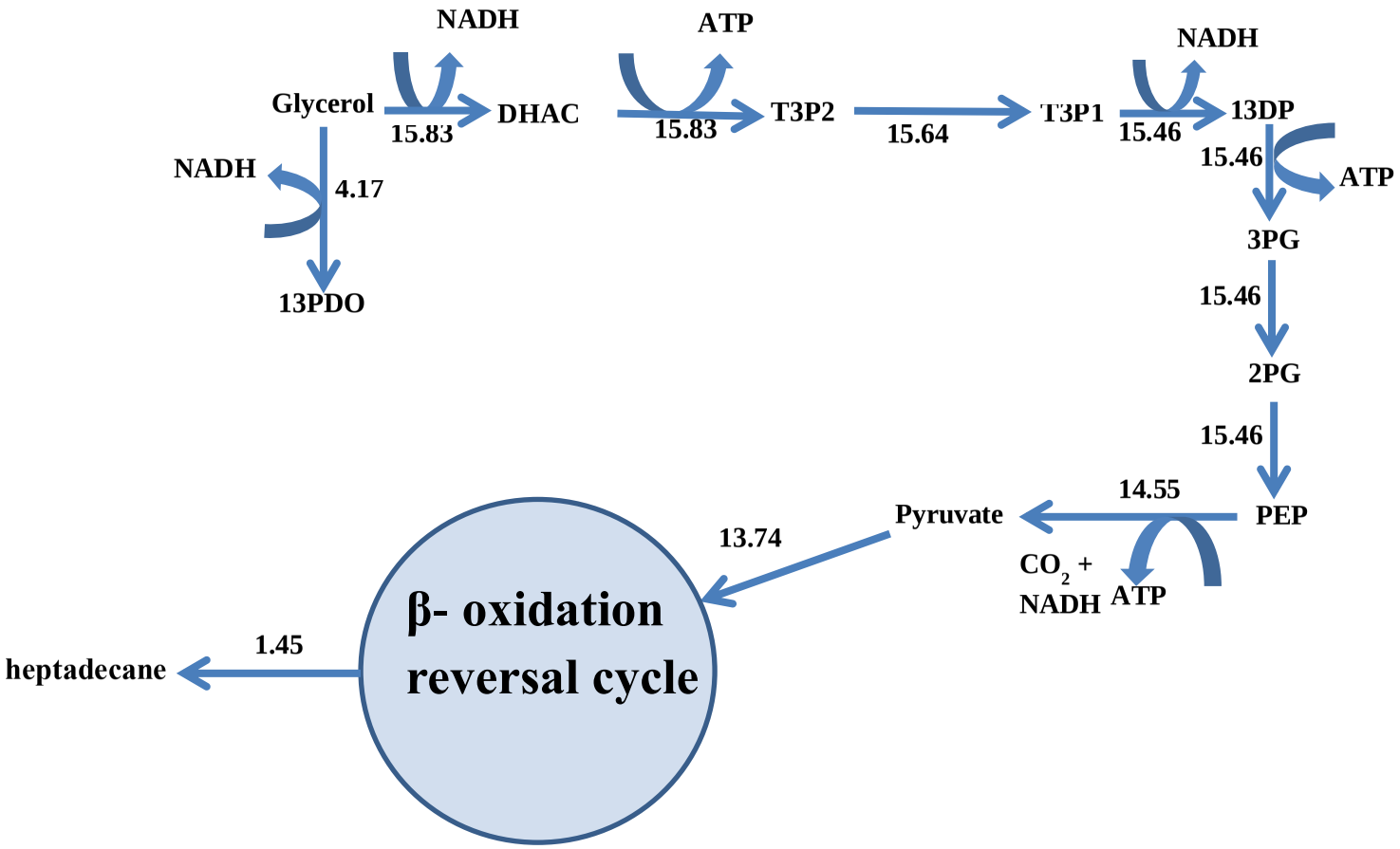


Figure 4.3 - Glycerol consuming optimum case flux map while producing heptadecane.

Appendix D

This section includes all the solution spaces obtained while producing alcohols, acids and alkanes using β-oxidation reversal cycle in *Zymomonas mobilis*.

4.3. Glucose

All the simulations in this section are performed in presence of glucose as the only carbon source after the removal or knock out of undesired metabolites. The knock outs involved major reactions producing acetate, succinate, ethanol, acetoin, lactate, malate and fumarate. The glucose uptake rate was fixed to an upper limit of 10 mmol$_{gCDw^{-1}}$ h^{-1}.There was no oxygen consumption in the model.

4.3.1. Acids

This section deals with simulation results of three types of acids produced with varying chain lengths – n-acids, enoic acids and hydroxy acids ;under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out; represented by Figure 4.4, Figure 4.5 and Figure 4.6 respectively.

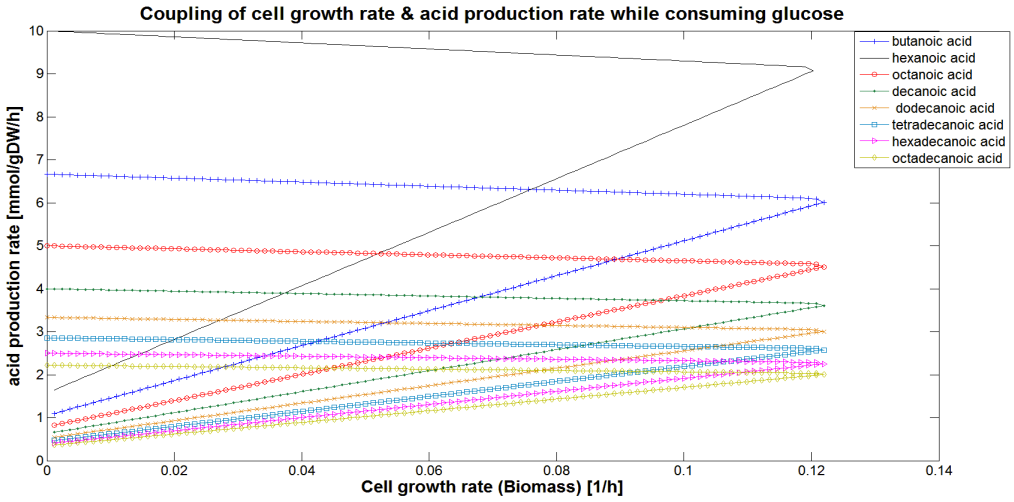


Figure 4.4 - *Solution space* of cell growth rate Vs. acid production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

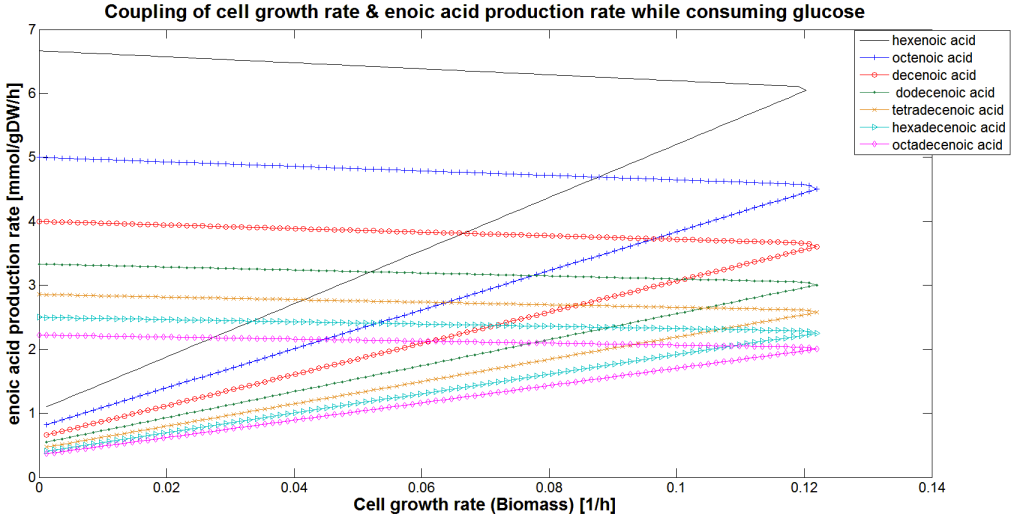


Figure 4.5 – *Solution space* of cell growth rate Vs. alkenoic acid production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length. The optimum growth rates show no relationship with carbon chain length.

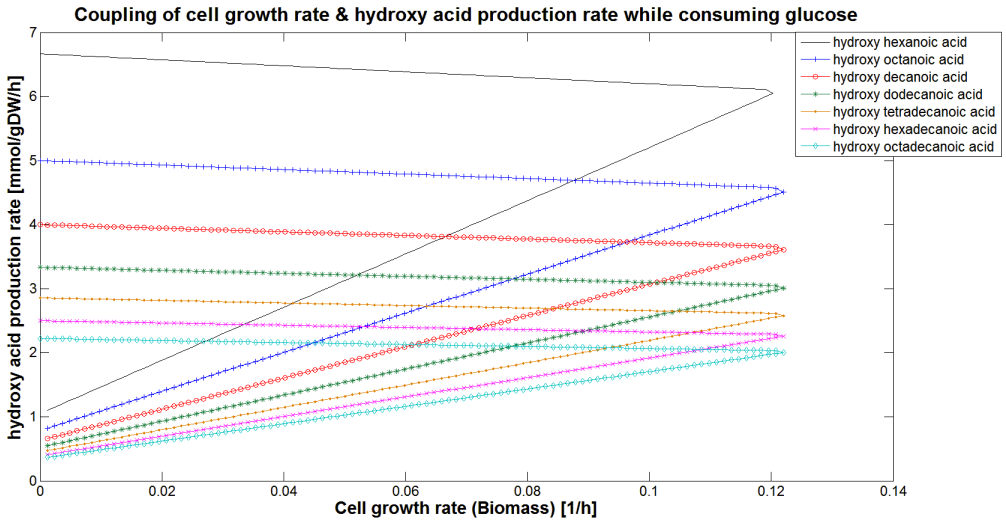


Figure 4.6 - *Solution space* of cell growth rate Vs. hydroxy acid production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

4.3.2. Alcohols

The following section deals with the four types of alcohols produced with varying chain lengths – n-alcohols, enoyl alcohols, hydroxy alcohols and oxo-alcohols ;under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out; represented by Figure 4.7, Figure 4.8, Figure 4.9 and Figure 4.10 respectively.

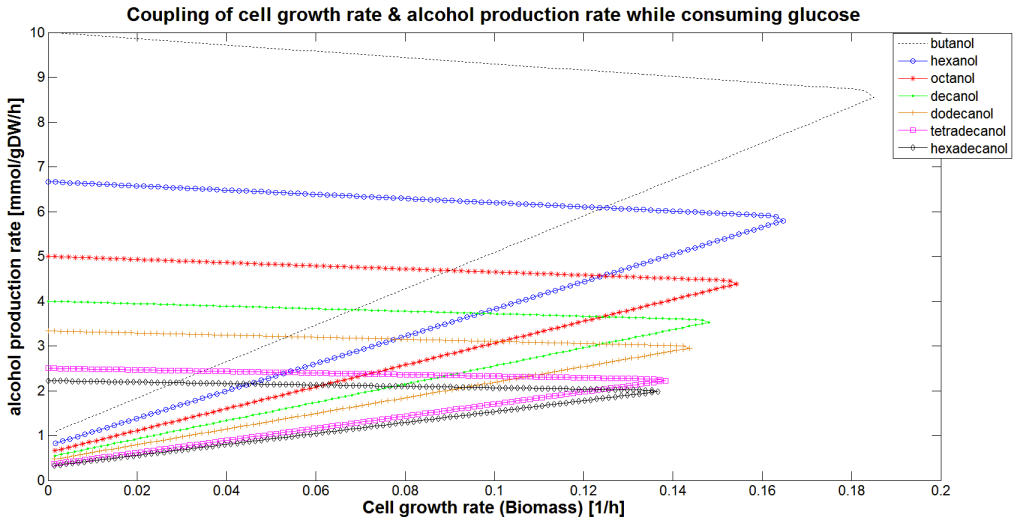


Figure 4.7- *Solution space* of cell growth rate Vs. alcohol production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show inverse relationship with carbon chain length; longer chain lengths grow lesser.

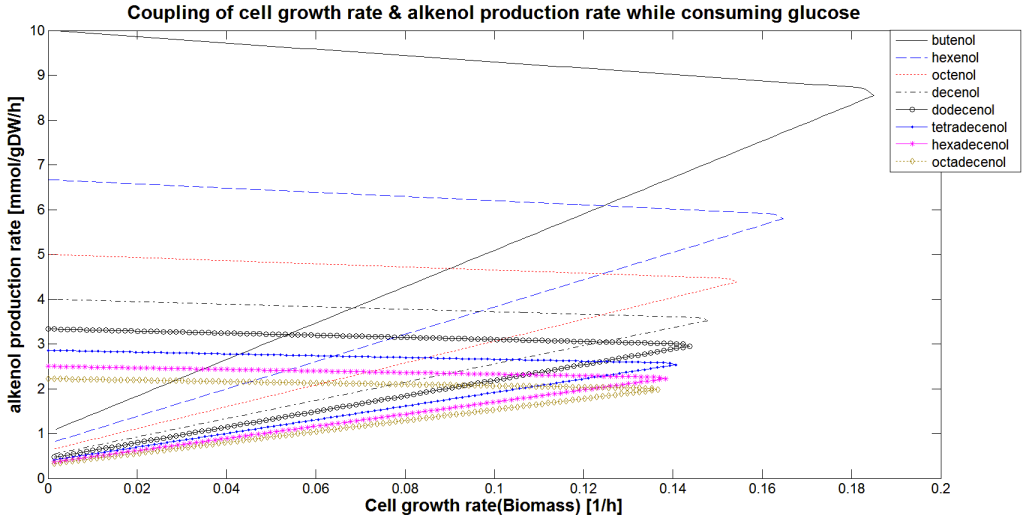


Figure 4.8 - *Solution space* of cell growth rate Vs. alkenol production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show inverse relationship with carbon chain length; longer chain lengths grow lesser.

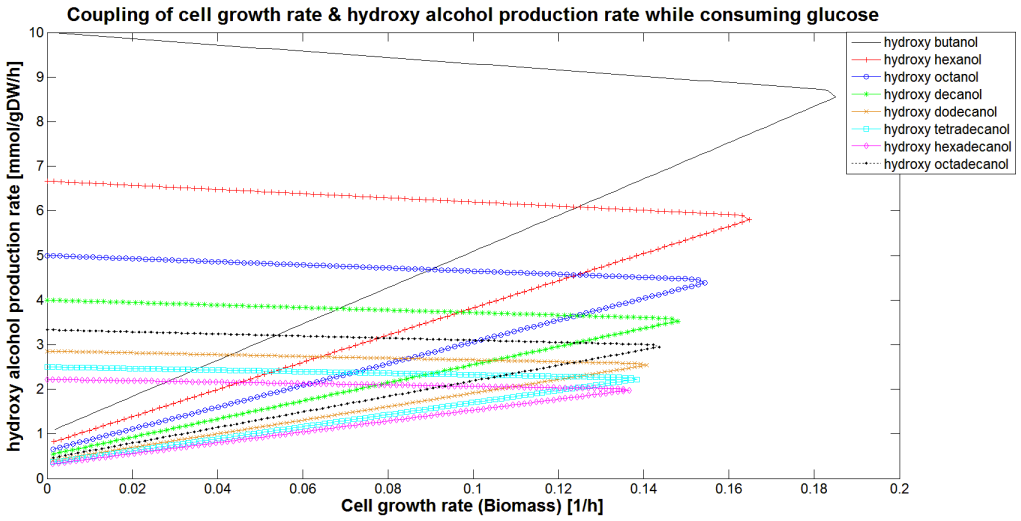


Figure 4.9 - *Solution space* of cell growth rate Vs. hydroxy alcohol production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show inverse relationship with carbon chain length; longer chain lengths grow lesser.

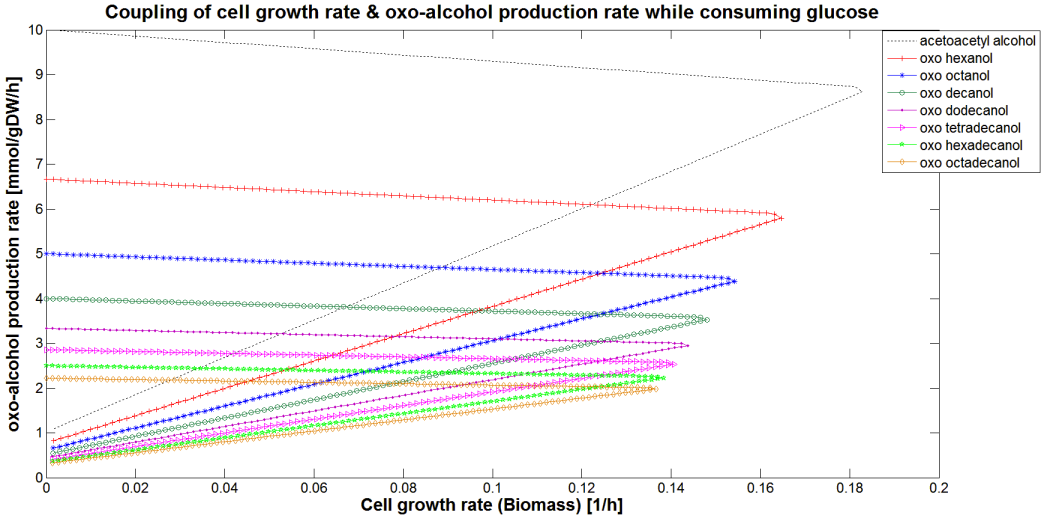


Figure 4.10 - *Solution space* of cell growth rate Vs. oxo-alcohol production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show inverse relationship with carbon chain length; longer chain lengths grow lesser.

All the four different alcohol groups namely n-alcohols, enoic-alcohols, hydroxy alcohols and oxo-alcohols show similar trend in solution space. There was coupling between cell growth rate and product formation rate. Also, the growth rate had an inverse relationship with carbon chain length. With increasing carbon chain length there was less growth. This has been investigated in the Discussion chapter.

4.3.3. Alkanes

The following section deals with the four types of alkanes produced with varying chain lengths – alkanes, alkenes, hydroxyl alkanes and oxo-alkanes ; under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out; represented by Figure 4.11, Figure 4.12, Figure 4.13 and Figure 4.14 respectively.

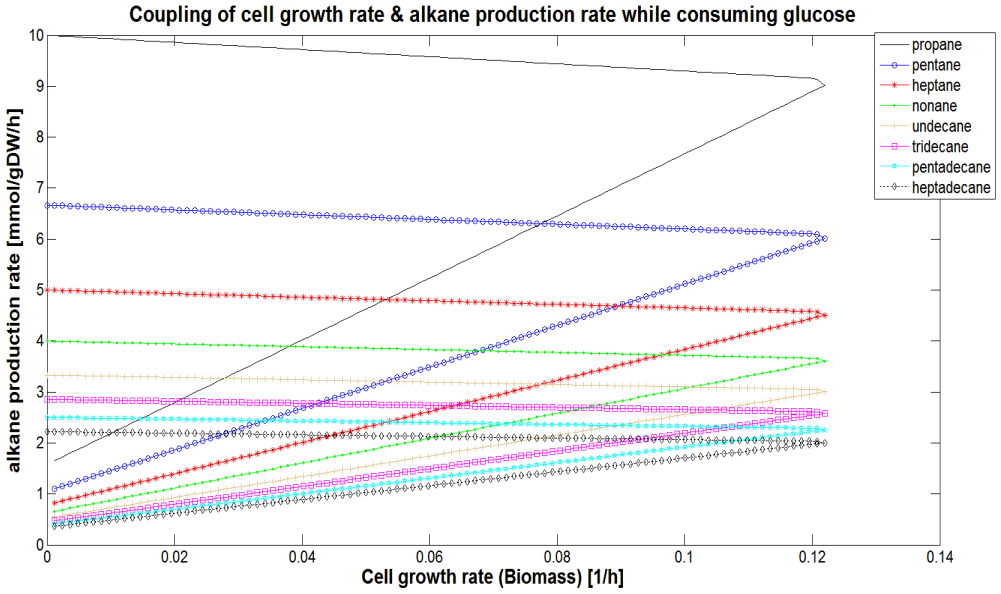


Figure 4.11 - *Solution space* of cell growth rate Vs. alkane production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

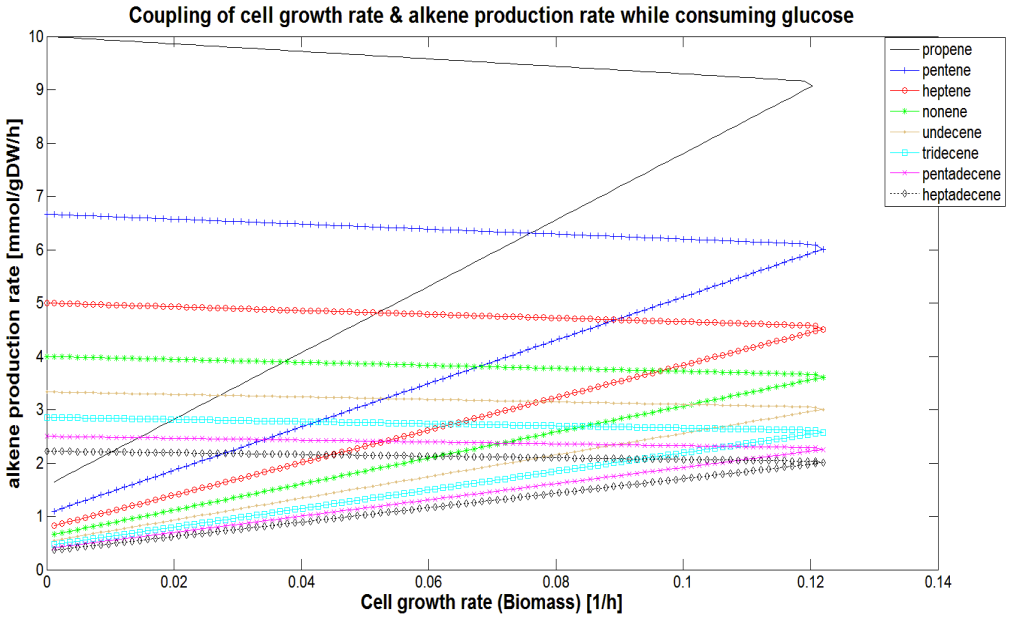


Figure 4.12 - *Solution space* of cell growth rate Vs. alkene production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

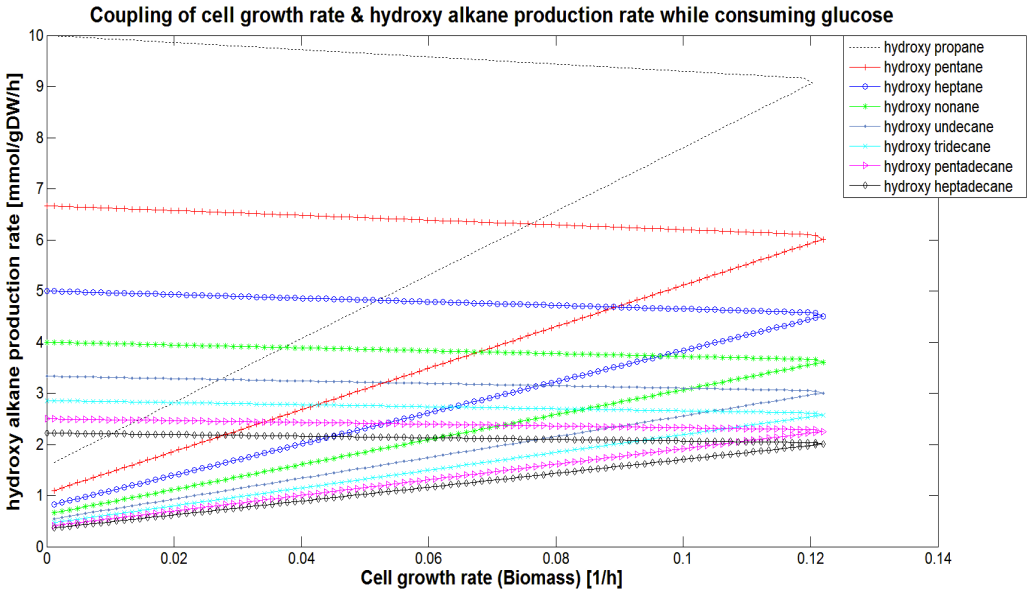


Figure 4.13 - *Solution space* of cell growth rate Vs. hydroxy alkane production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

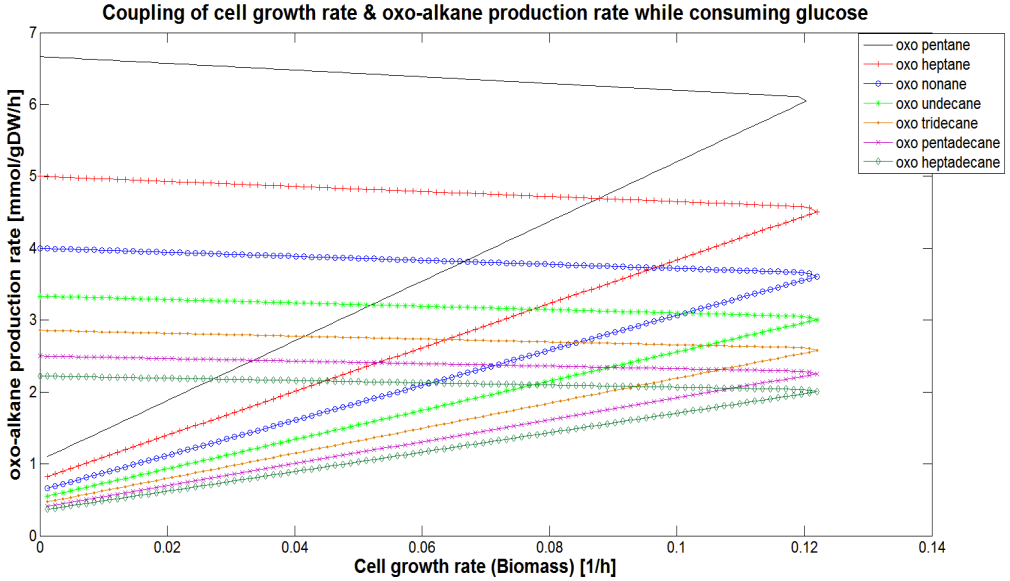


Figure 4.14- *Solution space* of cell growth rate Vs. oxo-alkane production rate using glucose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

All the four different alkane groups namely n-alkanes, alkenes, hydroxy alkanes and oxo-alkanes show similar trend in solution space. There was coupling between cell growth rate and product formation rate. Also, the growth rate was independent of carbon chain length. This has been investigated in the Discussion chapter.

4.4. Glycerol

All the simulations in this section are performed in presence of glycerol as the only carbon source after the removal or knock out of undesired metabolites. The knock outs involved major reactions producing acetate, fumarate, malate, succinate, acetoin, lactate and ethanol. The glycerol uptake rate was fixed to an upper limit of 20 $mmol_{gCDw^{-1}}\ h^{-1}$.There was no oxygen consumption in the model.

4.4.1. Acids

The following section deals with the three types of acids produced with varying chain lengths – n-acids, enoic acids and hydroxy acids ; under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out; represented by Figure 4.15, Figure 4.16 and Figure 4.17 respectively.

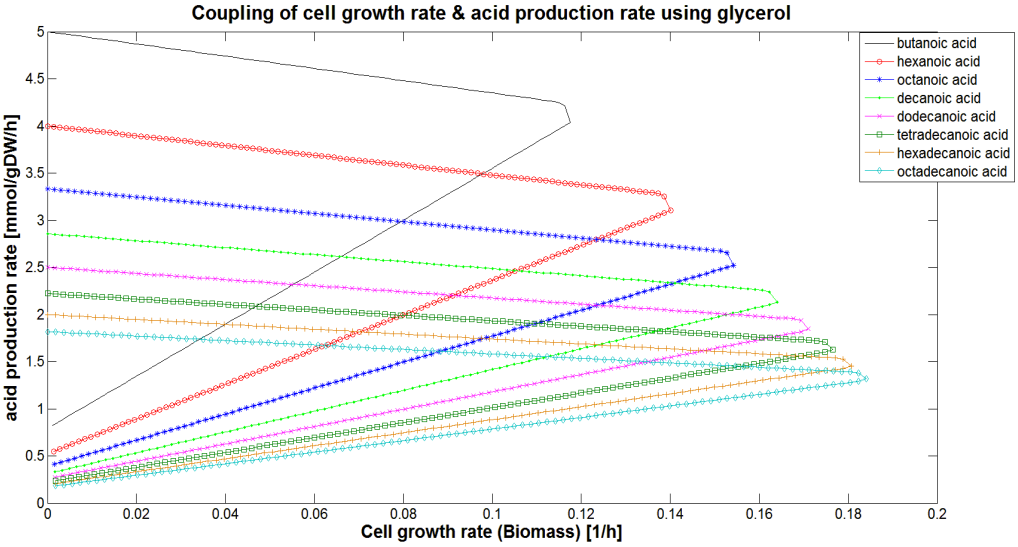


Figure 4.15 - *Solution space* of cell growth rate Vs. acid production rate using glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

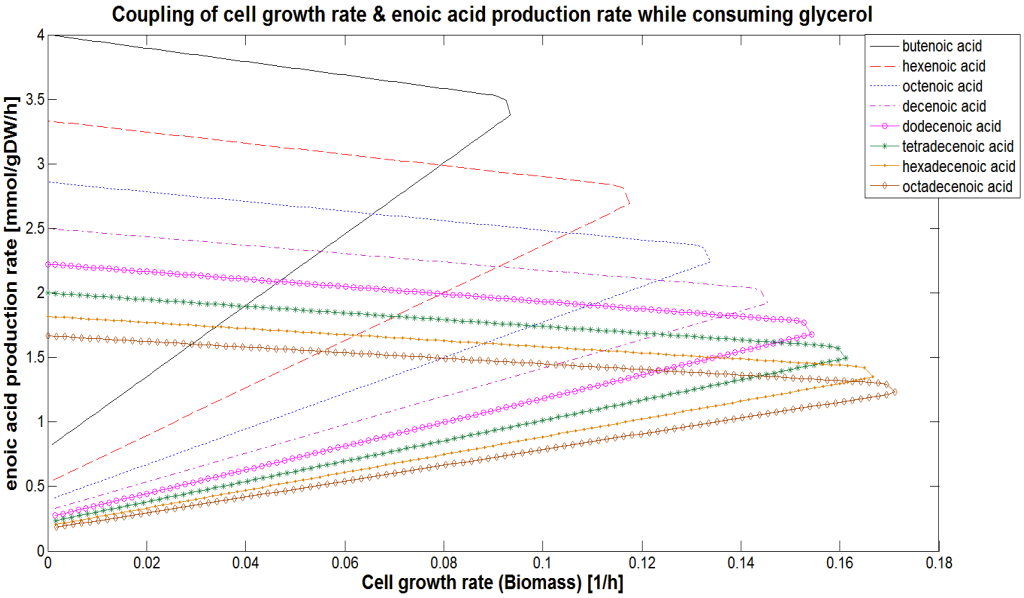


Figure 4.16 - *Solution space* of cell growth rate Vs. enoic acid production rate using glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

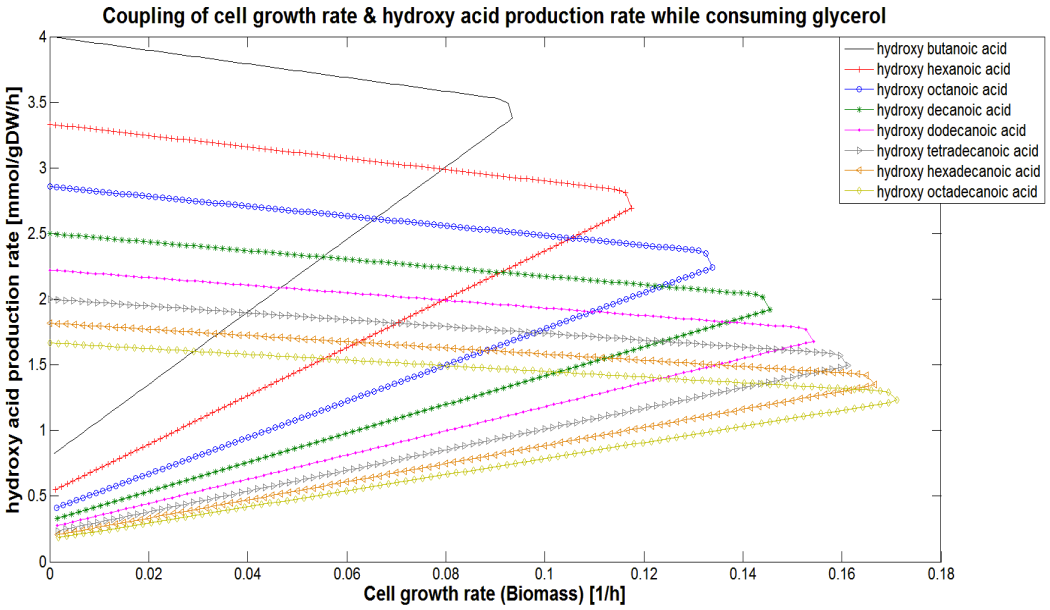


Figure 4.17 - *Solution space* of cell growth rate Vs. hydroxy acid production rate using glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

All the three different acids groups namely n-acids, enoic-acids and hydroxy acids show similar trend in ***solution space***. There was coupling between cell growth rate and product formation rate. Also, the growth rate had a positive co-relationship with carbon chain length. With increasing carbon chain length there was more growth. This has been investigated in the Discussion chapter.

4.4.2. Alcohols

The following section deals with the four types of alcohols produced with varying chain lengths – n-alcohols, enoyl alcohols, hydroxy alcohols and oxo-alcohols ; under anaerobic conditions with acetate, succinate, ethanol, acetoin,

lactate, malate and fumarate knocked out; represented by Figure 4.18, Figure 4.19, Figure 4.20 and Figure 4.21 respectively.

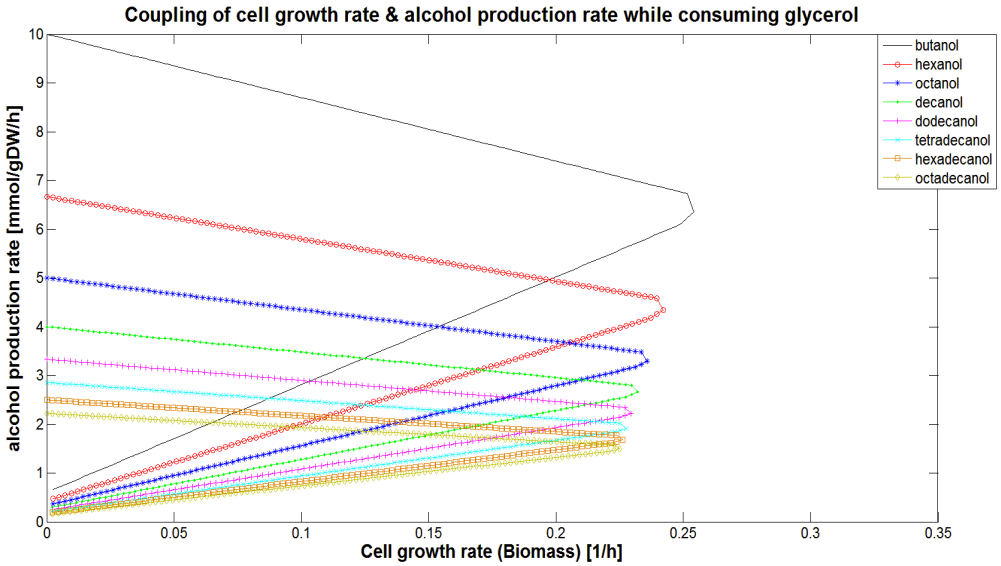


Figure 4.18 - *Solution space* of cell growth rate Vs. alcohol production rate using glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show inverse relationship with carbon chain length; longer chain lengths grow lesser.

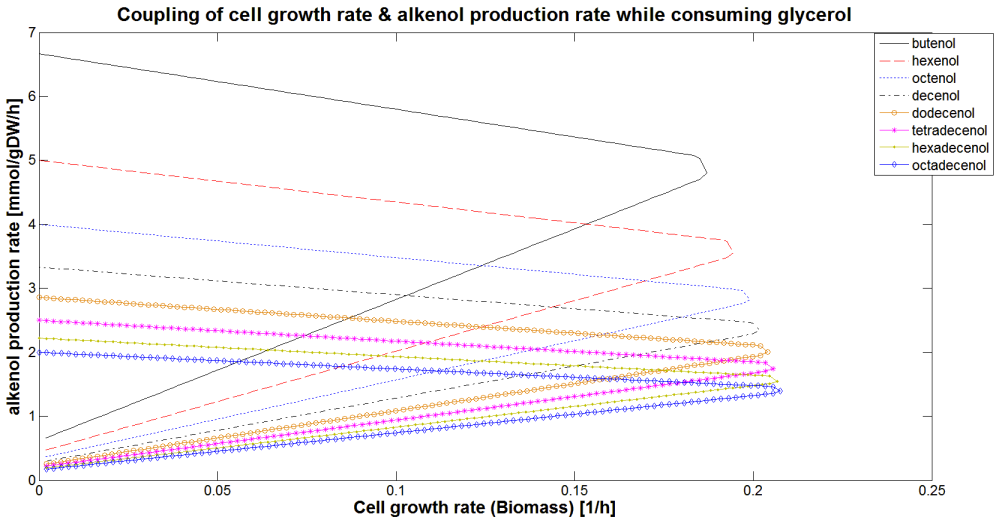


Figure 4.19 - *Solution space* of cell growth rate Vs. alkenol production rate using glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

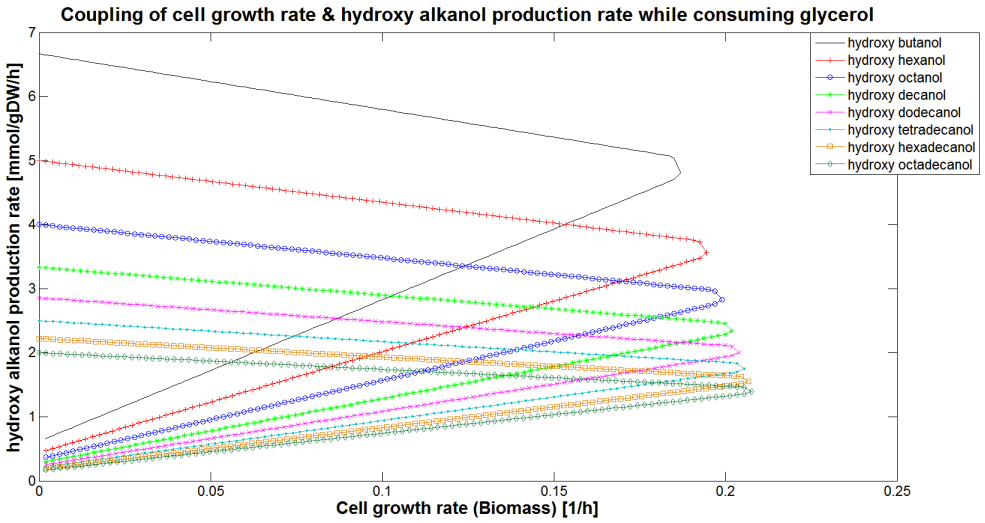


Figure 4.20- *Solution space* of cell growth rate Vs. hydroxy alcohol production rate using glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

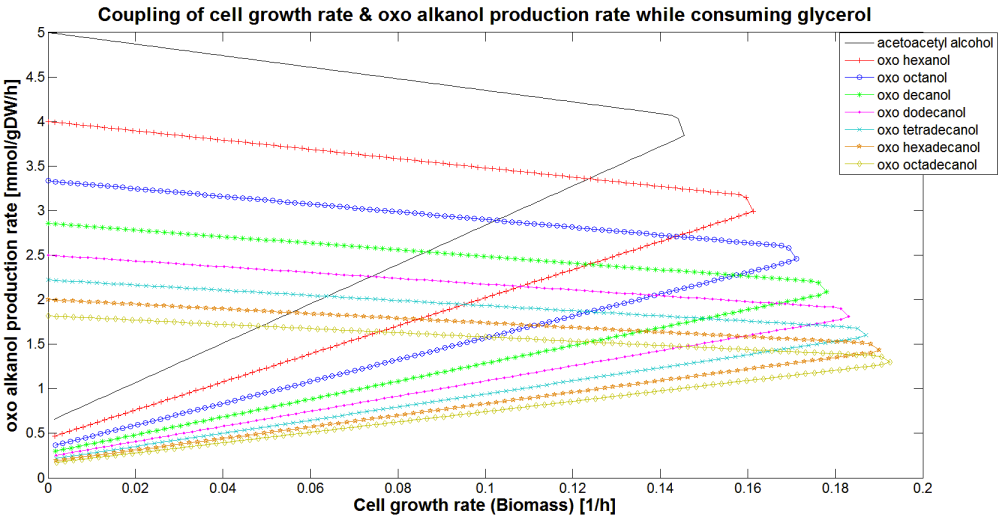


Figure 4.21- *Solution space* of cell growth rate Vs. oxo-alcohol production rate using glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

All the three different alcohol groups namely enoic-alcohols, hydroxy alcohols and oxo-alcohols show similar trend in ***solution space*** save n-alcohols. There was coupling between cell growth rate and product formation rate. Also, the growth rate had a positive co-relationship with carbon chain length for all except n-alcohols which showed the opposite trend. With increasing carbon chain length there was more growth except for the case of n-alcohols there was lesser growth. This has been investigated in the Discussion chapter.

4.4.3. Alkanes

The following section deals with the four types of alkanes produced with varying chain lengths – alkanes, alkenes, hydroxyl alkanes and oxo-alkanes ; under

anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out; represented by Figure 4.22, Figure 4.23, Figure 4.24and Figure 4.25 respectively.

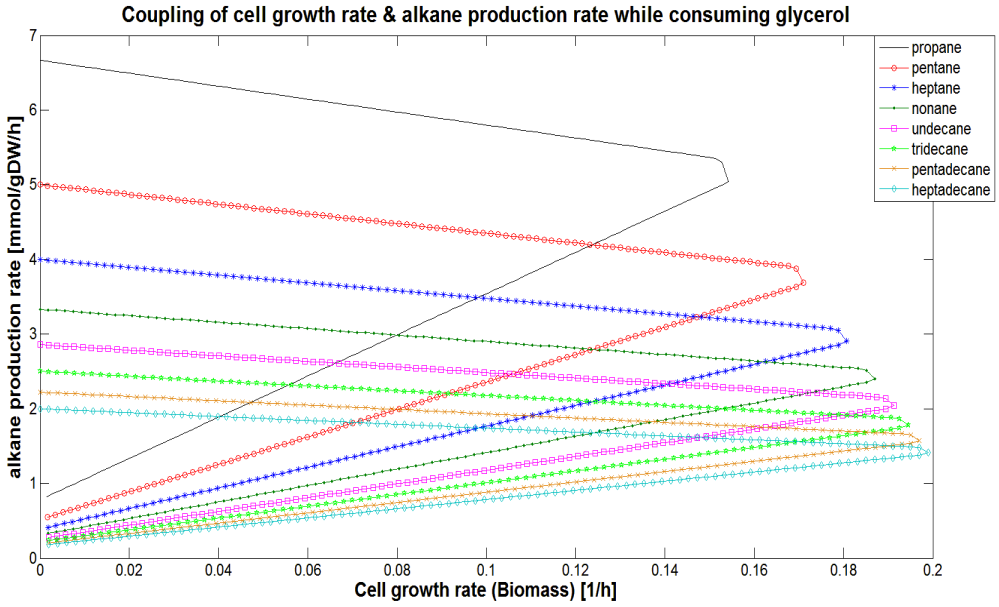


Figure 4.22 - *Solution space* of cell growth rate Vs. alkane production rate using glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

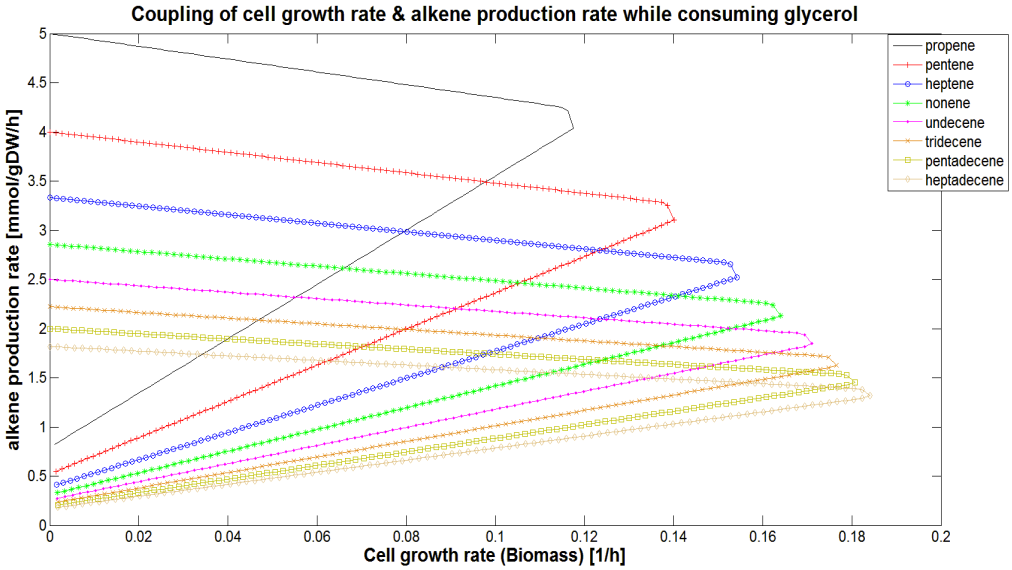


Figure 4.23 - *Solution space* of cell growth rate Vs. alkene production rate using glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

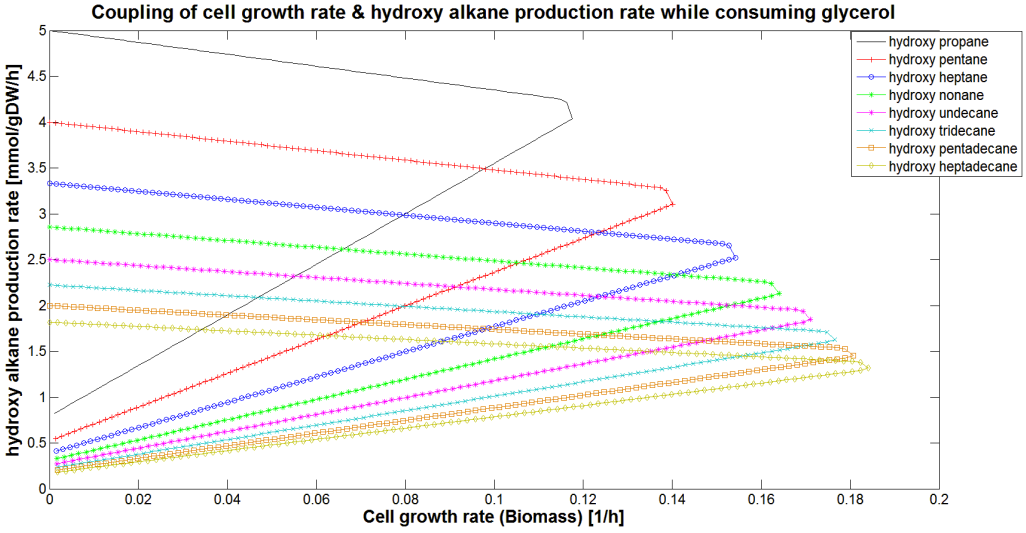


Figure 4.24 - *Solution space* of cell growth rate Vs. hydroxy alkane production rate using Glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

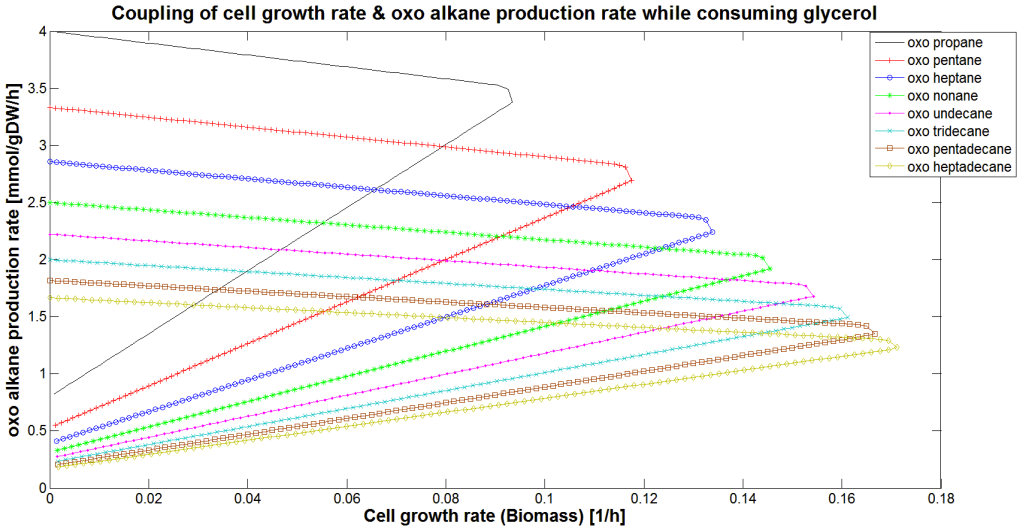


Figure 4.25 - *Solution space* of cell growth rate Vs. oxo-alkane production rateusing glycerol as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show positive relationship with carbon chain length; longer chain lengths grow more.

All the four different alkane groups namely n-alkanes, alkenes, hydroxy alkanes and oxo-alkanes show similar trend in ***solution space***. There was coupling between cell growth rate and product formation rate. Also, the growth rate had a positive co-relationship with carbon chain length. With increasing carbon chain length there was more growth. This has been investigated in the Discussion chapter.

4.5. Xylose & Arabinose

There is equivalency in consumption of either carbon source, due to presence of reactions converting intermediate from one source to another. Xylose is considered in this study and the results hence obtained can be extended for

arabinose as well. All the simulations in this section are performed in presence of xylose/arabinose as the only carbon source after the removal or knock out of undesired metabolites. The knock outs involved major reactions producing acetate, succinate, ethanol, acetoin, lactate, malate and fumarate. There are reactions which inter convert xylose and arabinose derived metabolites ensuring equivalent result in either case. The xylose uptake rate was fixed to an upper limit of 12 $mmol gCDw^{-1}\ h^{-1}$.There was no oxygen consumption in the model.

4.5.1. Acids

The following section deals with the three types of acids produced with varying chain lengths – n-acids, enoic acids and hydroxy acids; under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out; represented by Figure 4.26, Figure 4.27 and Figure 4.28 respectively.

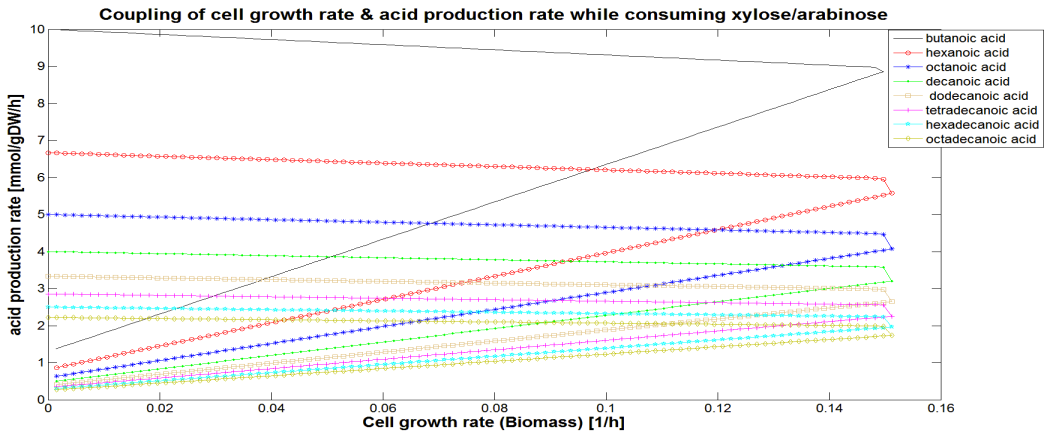


Figure 4.26 - *Solution space* of cell growth rate Vs. acid production rate with varying carbon cahin length using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate all knocked out. The optimum growth rates show no relationship with carbon chain length.

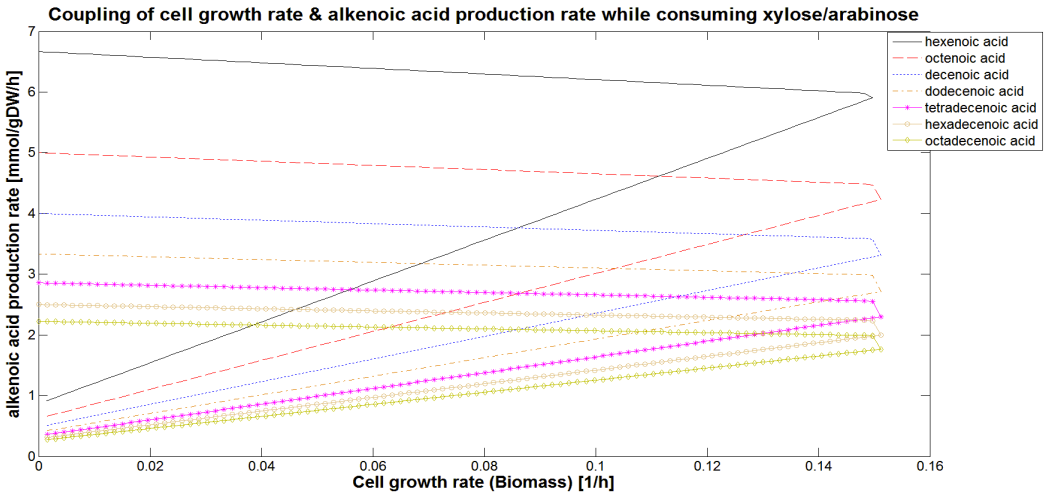


Figure 4.27 - *Solution space* of cell growth rate Vs. enoic acid production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

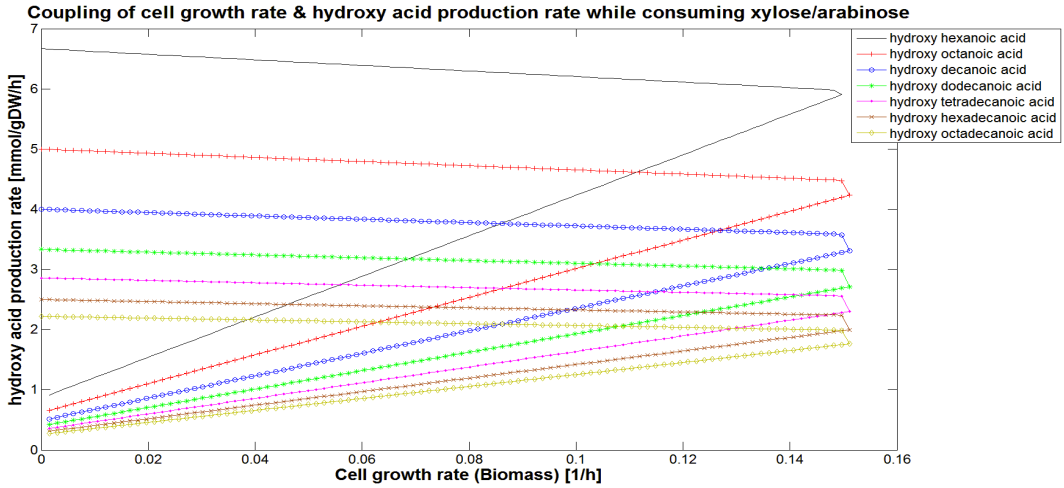


Figure 4.28 - *Solution space* of cell growth rate Vs. hydroxy acid production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

All the three different alcohol groups namely n-acids, enoic-acids and hydroxy acids show similar trend in ***solution space***. There was coupling between cell growth rate and product formation rate. Also, the growth rate was independent of carbon chain length. This has been investigated in the Discussion chapter.

4.5.2. Alcohols

The following section deals with the four types of alcohols produced with varying chain lengths – n-alcohols, enoyl alcohols, hydroxy alcohols and oxo-alcohols; under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out; represented by Figure 4.29, Figure 4.30, Figure 4.31 and Figure 4.32 respectively.

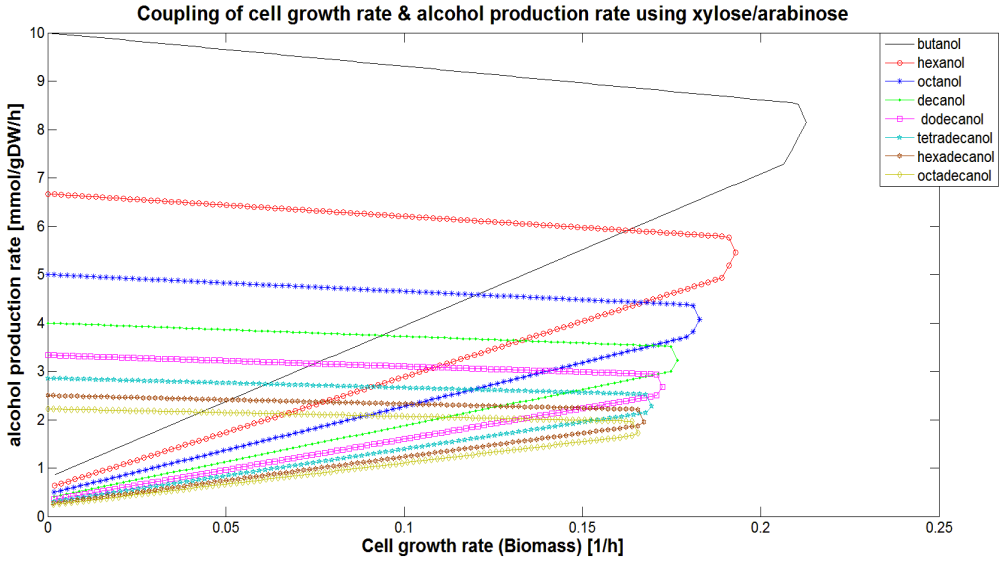


Figure 4.29 - *Solution space* of cell growth rate Vs. alcohol production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show inverse relationship with carbon chain length; longer chain lengths grow lesser.

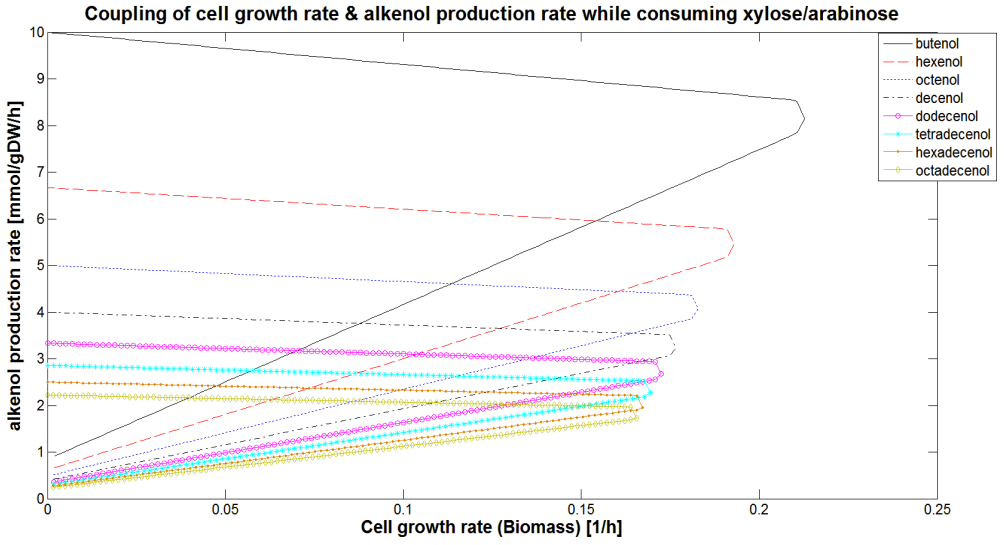


Figure 4.30- *Solution space* of cell growth rate Vs. alkenol production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show inverse relationship with carbon chain length; longer chain lengths grow lesser.

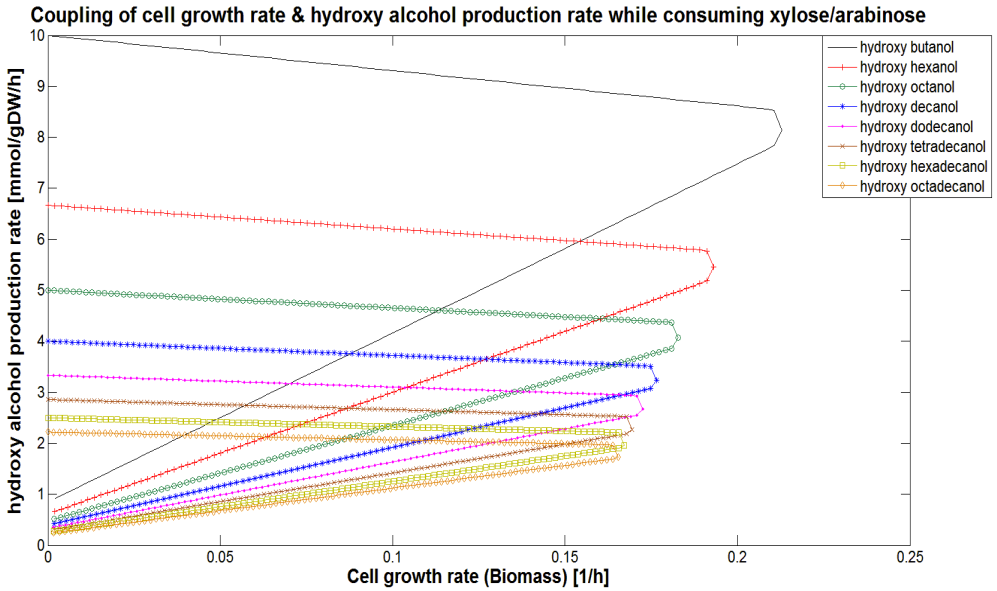


Figure 4.31 - *Solution space* of cell growth rate Vs. hydorxy alcohol production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show inverse relationship with carbon chain length; longer chain lengths grow lesser.

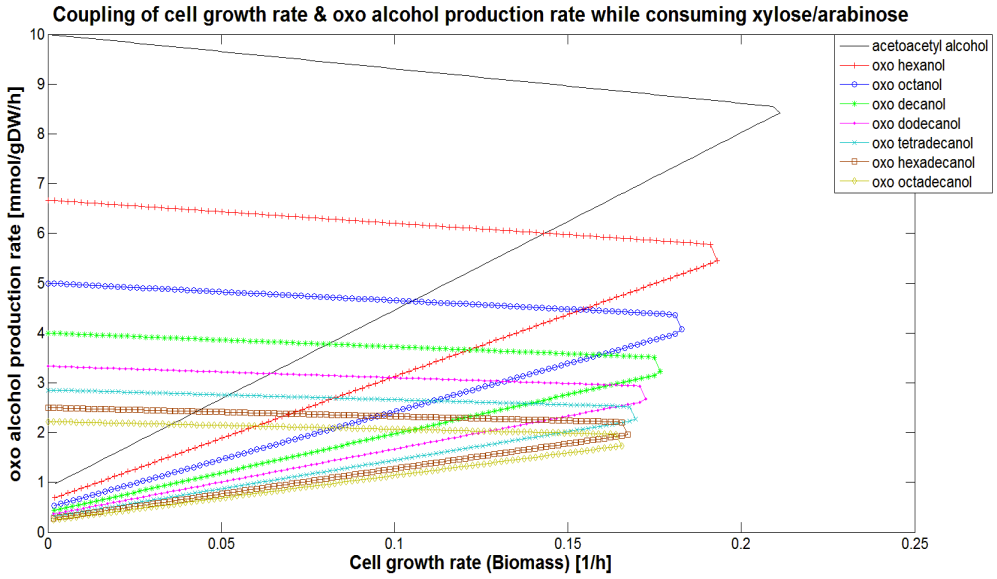


Figure 4.32 - *Solution space* of cell growth rate Vs. oxo-alcohol production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show inverse relationship with carbon chain length; longer chain lengths grow lesser.

All the four different alcohol groups namely n-alcohols, enoic-alcohols, hydroxy alcohols and oxo-alcohols show similar trend in ***solution space***. There was coupling between cell growth rate and product formation rate. Also, the growth rate had an inverse relationship with carbon chain length. With increasing carbon chain length there was less growth. This has been investigated in the Discussion chapter.

4.5.3. Alkanes

The following section deals with the four types of alkanes produced with varying chain lengths – alkanes, alkenes, hydroxyl alkanes and oxo-alkanes; under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out; represented by Figure 4.33, Figure 4.34, Figure 4.35 and Figure 4.36 respectively.

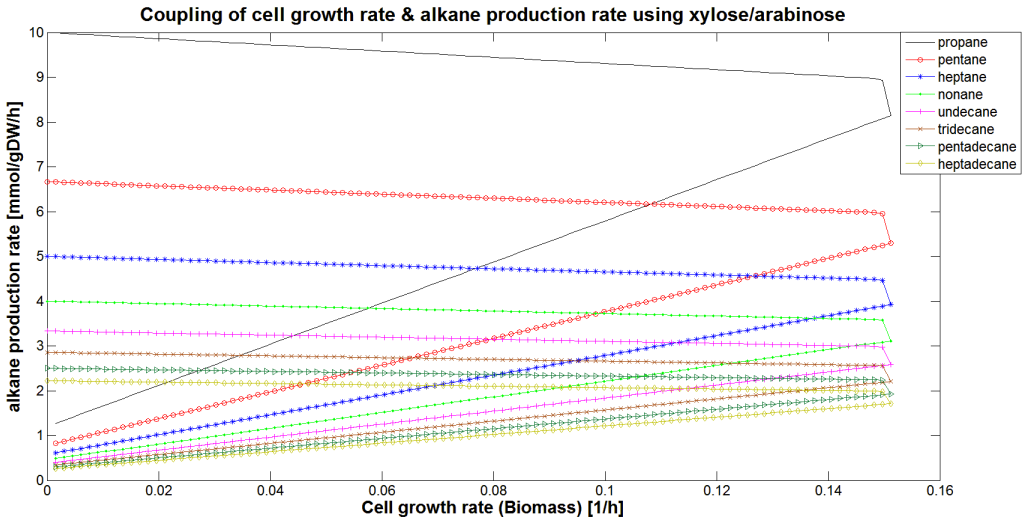


Figure 4.33 - Solution space of cell growth rate Vs. alkane production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

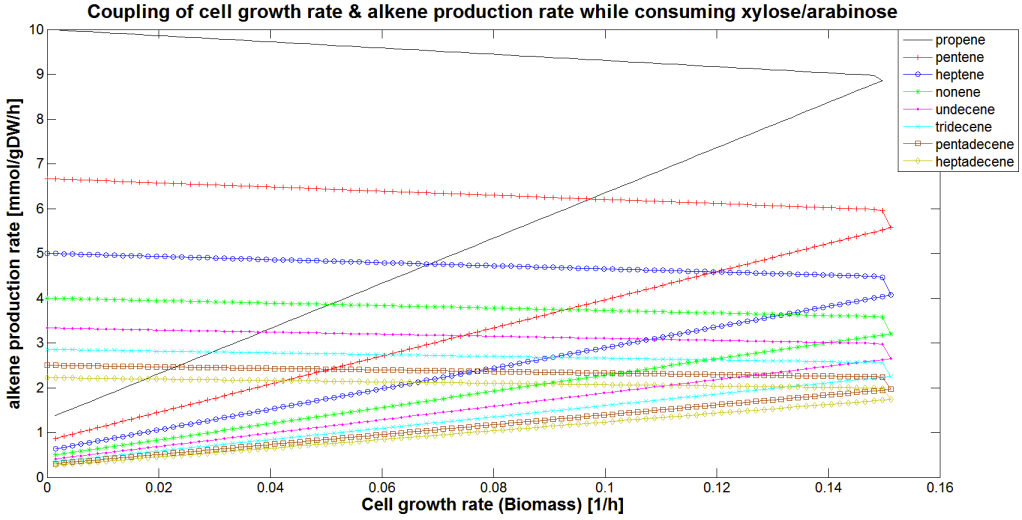


Figure 4.34 - Solution space of cell growth rate Vs. alkene production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

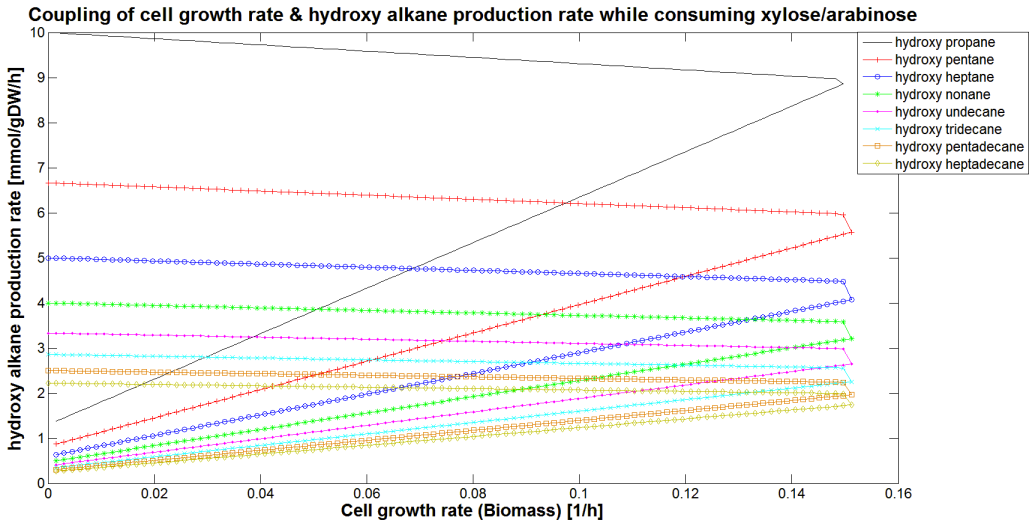


Figure 4.35 - Solution space of cell growth rate Vs. hydroxy alkane production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

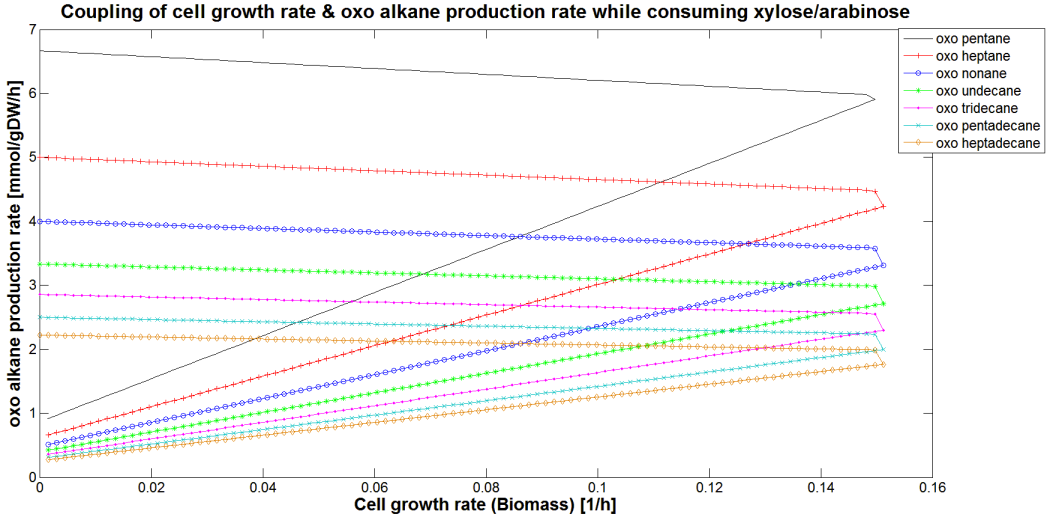


Figure 4.36 - Solution space of cell growth rate Vs. oxo-alkane production rate using xylose/arabinose as carbon source under anaerobic conditions with acetate, succinate, ethanol, acetoin, lactate, malate and fumarate knocked out. The optimum growth rates show no relationship with carbon chain length.

All the four different alkane groups namely n-alkanes, alkenes, hydroxy alkanes and oxo-alkanes show similar trend in ***solution space***. There was coupling between cell growth rate and product formation rate. Also, the growth rate was independent of carbon chain length. This has been investigated in the Discussion chapter.

Appendix E

This section includes the code used in Matlab R2010a to perform simulations using COBRA v2.0.

```
clear model
model = createModel({},{},{});
model = addReaction(model,'R001', 'G6P <=> bDG6P');
model = addReaction(model,'R002', 'G6P <=> F6P');
model = addReaction(model,'R003', 'bDG6P <=> F6P');
model = addReaction(model,'R004', 'GLC + ATP <=> G6P + ADP');
model = addReaction(model,'R005', 'bDGLC <=> GLC');
model = addReaction(model,'R006', 'FDP -> F6P + PI');
model = addReaction(model,'R007', 'FDP <=> T3P1 + T3P2');
model = addReaction(model,'R008', 'T3P1 <=> T3P2');
model = addReaction(model,'R009', 'T3P1 + PI + NAD <=> NADH + 13DPG +
H');
model = addReaction(model,'R010', '13DPG + ADP <=> 3PG + ATP');
model = addReaction(model,'R011', '3PG <=> 2PG');
model = addReaction(model,'R012', '2PG <=> PEP');
model = addReaction(model,'R013', 'PEP + ADP -> PYR + ATP');%could do
without this
model = addReaction(model,'R014', 'PYR + THPP -> 2HETHPP + CO2');
model = addReaction(model,'R015', '2HETHPP + LIPO -> ADLIPO + THPP');
model = addReaction(model,'R016', 'COA + ADLIPO -> DLIPO + ACCOA');
model = addReaction(model,'R017', 'DLIPO + NAD -> LIPO + NADH + H');
model = addReaction(model,'R018', 'PYR -> ACAL + CO2');
model = addReaction(model,'R019', 'ACAL + NADH + H <=> ETH + NAD');
model = addReaction(model,'R020', 'G6P <=> G1P');
model = addReaction(model,'R021', 'bDGLC + ATP -> bDG6P + ADP');
model = addReaction(model,'R022', 'ACCOA + OA -> COA + CIT');
model = addReaction(model,'R023', 'CIT <=> AC + OA');
model = addReaction(model,'R024', 'CIT <=> ICIT');
model = addReaction(model,'R025', 'ICIT + NADP <=> CO2 + NADPH + AKG +
H');
model = addReaction(model,'R026', 'FUM <=> MAL');
model = addReaction(model,'R027', 'FUM + FADH2 <=> SUCC + FAD');
model = addReaction(model,'R028', 'SUCCOA + ADP + PI <=> ATP + COA +
SUCC');
model = addReaction(model,'R029', 'GLCNDL -> GLCN');
model = addReaction(model,'R030', 'ATP + GLCN -> ADP + D6PGC');
model = addReaction(model,'R031', '2KD6PG -> T3P1 + PYR');
model = addReaction(model,'R032', 'D6PGC -> 2KD6PG');
model = addReaction(model,'R033', 'D6PGL -> D6PGC');
model = addReaction(model,'R034', 'D6PGC + NADP -> NADPH + CO2 + RL5P +
H');
model = addReaction(model,'R035', 'bDG6P + NADP <=> D6PGL + NADPH +
H');
model = addReaction(model,'R036', 'X5P + E4P <=> F6P + T3P1');
model = addReaction(model,'R037', 'R5P + X5P <=> T3P1 + S7P');
```

```
model = addReaction(model,'R038', 'RL5P <=> X5P');
model = addReaction(model,'R039', 'RL5P <=> R5P');
model = addReaction(model,'R040', 'R5P <=> R1P');
model = addReaction(model,'R041', 'GLUC + NADP <=> 2kGLUC + NADPH +
H');
model = addReaction(model,'R042', 'UDPG + 2 NAD <=> UDPGLC + 2 NADH + 2
H');
model = addReaction(model,'R043', 'UTP + G1P <=> PPI + UDPG');
model = addReaction(model,'R044', 'SOB + FADH2 -> SOT + FAD');
model = addReaction(model,'R045', 'MAN6P <=> MAN1P');
model = addReaction(model,'R046', 'MAN6P <=> F6P');
model = addReaction(model,'R047', 'F1P <=> T3P1 + T3P2');
model = addReaction(model,'R048', 'S6P + NADP <=> SB1P + NADPH + H');
model = addReaction(model,'R049', 'LCTS -> GLAC + GLC');
model = addReaction(model,'R050', 'RAF -> MELI + FRU');
model = addReaction(model,'R051', 'ST -> G6GG + FRU');
model = addReaction(model,'R052', 'g14l <=> guln');
model = addReaction(model,'R053', 'SUC -> GLC + LEVAN');
model = addReaction(model,'R054', 'SUC -> FRU + GLC');
model = addReaction(model,'R055', 'ATP + FRU <=> ADP + F6P');
model = addReaction(model,'R056', 'ATP + G1P -> ADPGLC + PPI');
model = addReaction(model,'R057', 'ADPGLC -> ADP + GLYCOGEN');
model = addReaction(model,'R058', 'GLYCOGEN + PI -> G1P');
model = addReaction(model,'R059', 'SUC6P -> FRU + G6P');
model = addReaction(model,'R060', 'GA6P + AC <=> NAGP');
model = addReaction(model,'R061', 'UTP + NAG1P <=> PPI + UDPNAG');
model = addReaction(model,'R062', 'ACCOA + GA1P -> COA + NAG1P');
model = addReaction(model,'R063', 'GA6P -> GA1P');
model = addReaction(model,'R064', 'GLN + F6P <=> GLU + GA6P');
model = addReaction(model,'R065', 'PEP + UDPNAG -> UDPNAGEP + PI');
model = addReaction(model,'R066', 'UDPNAGEP + NADPH + H -> UDPNAM +
NADP');
model = addReaction(model,'R067', 'CTB -> 2 NAGA');
model = addReaction(model,'R068', 'UDPNAGEP + NADH + H -> UDPNAM +
NAD');
model = addReaction(model,'R069', 'UDPGLC -> UDPIDU');
model = addReaction(model,'R070', 'ACMU6P <=> NAGP + LAC');
model = addReaction(model,'R071', 'RGT + MTGYX <=> SLGT');
model = addReaction(model,'R072', 'SLGT <=> RGT + LAC');
model = addReaction(model,'R073', 'PYR + NADH + H <=> NAD + LAC');
model = addReaction(model,'R074', 'PEP + CO2 -> OA + PI');
model = addReaction(model,'R075', 'PYR + COA -> ACCOA + FORT');
model = addReaction(model,'R076', 'MAL + NAD <=> CO2 + NADH + PYR +
H');
model = addReaction(model,'R077', 'ACCOA + ATP + CO2 -> MALCOA + ADP +
PI');
model = addReaction(model,'R078', 'NDP + PEP -> NTP + PYR');
model = addReaction(model,'R079', '2PG -> GLYCOLATE + PPI');
model = addReaction(model,'R080', 'GLYCOAL + NADP <=> GLYCOLATE + NADPH
+ H');
model = addReaction(model,'R081', 'FORGLU -> FORT + GLU');
model = addReaction(model,'R082', 'PYR + GLX <=> HYDROXYAKG');
model = addReaction(model,'R083', 'OBUT + COA <=> PPACOA + FORT');
model = addReaction(model,'R084', 'TPP + PYR -> HTPP + CO2');
model = addReaction(model,'R085', 'SUCCSAL + NAD -> SUCC + NADH + H');
```

```
model = addReaction(model,'R086', 'SUCCSAL + NADP -> SUCC + NADPH +
H');
model = addReaction(model,'R087', 'BUTAL + NADH + H <=> 1BOH + NAD');
model = addReaction(model,'R088', 'BUTAL + NADPH + H <=> 1BOH + NADP');
model = addReaction(model,'R089', '2 PYR -> ACLAC + CO2');
model = addReaction(model,'R090', 'ACLAC -> ACTN + CO2');
model = addReaction(model,'R091', 'ATP + ITCN + COA <=> ADP + PI +
ITCNCOA');
model = addReaction(model,'R092', 'I3P -> INSTOL + PPI');
model = addReaction(model,'R093', 'CDPDG + INSTOL -> CMP + PINSTOL');
model = addReaction(model,'R094', 'I4P -> INSTOL + PPI');
model = addReaction(model,'R095', 'IP -> INSTOL + PPI');
model = addReaction(model,'R096', 'IHKP -> IPKP + PI');
model = addReaction(model,'R097', 'QH2 + ccfe3 <=> Q + ccfe2 + 4
HEXT'); % changing  4 INTO 2
model = addReaction(model,'R098', 'PPI -> 2 PI');
model = addReaction(model,'R099', 'ATP + PPI <=> ADP + PPPI');
model = addReaction(model,'R100', 'ATP + 2 H <=> ADP + PI + 2
HEXT');%removing 2
model = addReaction(model,'R101', 'RFEDX + NADP  + H <=> OFRDX +
NADPH');
model = addReaction(model,'R102', 'FUM + QH2 <=> SUCC + Q');
model = addReaction(model,'R103', 'QH2 + 2 FAD -> Q + 2 FADH2 + 2 HEXT'
);%removing 2 HEXT% need to check
model = addReaction(model,'R104', 'NADH + Q + H-> NAD + QH2');%
removing 2 HEXT
model = addReaction(model,'R105', 'NADPH + NAD <=> NADP + NADH');
model = addReaction(model,'R106', 'NADH + 0.5 O2 + H -> NAD');
model = addReaction(model,'R107', 'H2O2 + METHANOL -> FALD');
model = addReaction(model,'R108', 'METTHF + NADH + H <=> MTHF + NAD');
%!!?? ADD H?!%change to NAD
model = addReaction(model,'R109', 'HMGT + NAD -> FMGT + NADH + H');
model = addReaction(model,'R110', 'H2CO3 <=> CO2');
model = addReaction(model,'R111', 'GLN + AKG + NADPH + H -> 2 GLU +
NADP');
model = addReaction(model,'R112', 'ATP + GLU + NH3 <=> ADP + PI +
GLN');
model = addReaction(model,'R113', '8 RFEDX + 16 ATP + N2 + 8 H -> 8
OFRDX + 16 PI + 16 ADP + 2 NH3');
model = addReaction(model,'R114', 'GLN + AKG + NADH + H -> 2 GLU +
NAD');
model = addReaction(model,'R115', 'RCN -> RCO2 + NH3');
model = addReaction(model,'R116', 'ATP + SLF -> PPI + APS');
model = addReaction(model,'R117', 'ATP + APS <=> ADP + PAPS');
model = addReaction(model,'R118', 'PAPS + RTHIO -> PAP + H2SO3 +
OTHIO');
model = addReaction(model,'R119', 'H2SO3 + 3 NADPH + 3 H -> H2S + 3
NADP');
model = addReaction(model,'R120', 'ACCOA + HSER <=> COA + OAHSER');
model = addReaction(model,'R121', 'LLCT -> HCYS + PYR + NH3');
model = addReaction(model,'R122', 'PAP -> AMP + PI');
model = addReaction(model,'R123', 'CYS + OAHSER -> LLCT + AC');
model = addReaction(model,'R124', 'MALCOA + ACP <=> MALACP + COA');
model = addReaction(model,'R125', 'ACACP + COA <=> ACCOA + ACP');
```

```
model = addReaction(model,'R126', 'ACACP + 5 MALACP + 10 NADPH + 10 H -
> 10 NADP + C120ACP + 5 CO2 + 5 ACP');
model = addReaction(model,'R127', 'ACACP + 6 MALACP + 12 NADPH + 12 H -
> 12 NADP + C140ACP + 6 CO2 + 6 ACP');
model = addReaction(model,'R128', 'ACACP + 6 MALACP + 11 NADPH + 11 H -
> 11 NADP + C141ACP + 6 CO2 + 6 ACP');
model = addReaction(model,'R129', 'ACACP + 7 MALACP + 14 NADPH + 14 H -
> 14 NADP + C160ACP + 7 CO2 + 7 ACP');
model = addReaction(model,'R130', 'ACACP + 7 MALACP + 13 NADPH + 13 H -
> 13 NADP + C161ACP + 7 CO2 + 7 ACP');
model = addReaction(model,'R131', 'ACACP + 8 MALACP + 16 NADPH + 16 H -
> 16 NADP + C180ACP + 8 CO2 + 8 ACP');
model = addReaction(model,'R132', 'ACACP + 8 MALACP + 15 NADPH + 15 H -
> 15 NADP + C181ACP + 8 CO2 + 8 ACP');
model = addReaction(model,'R133', 'C181ACP -> C190ACP');
model = addReaction(model,'R134', 'NADPH + T3P2 + H <=> GL3P + NADP');
model = addReaction(model,'R135', 'GL3P + 0.007 C120ACP + 0.42 C140ACP
+ 0.007 C141ACP + 0.254 C160ACP + 0.175 C161ACP + 0.013 C180ACP + 0.112
C181ACP -> AGL3P + ACP');
model = addReaction(model,'R136', 'AGL3P + 0.007 C120ACP + 0.42 C140ACP
+ 0.007 C141ACP + 0.254 C160ACP + 0.175 C161ACP + 0.013 C180ACP + 0.112
C181ACP -> PA + ACP');
model = addReaction(model,'R137', 'PA + CTP <=> CDPDG + PPI');
model = addReaction(model,'R138', 'CDPDG + SER -> CMP + PS');
model = addReaction(model,'R139', 'CDPDG + GL3P <=> CMP + PGP');
model = addReaction(model,'R140', 'CDPDG + PG <=> CMP + CL');
model = addReaction(model,'R141', 'PGP -> PI + PG');
model = addReaction(model,'R142', 'PS -> PE + CO2');
model = addReaction(model,'R143', 'SAM + PE -> SAH + PNME');
model = addReaction(model,'R144', 'SAM + PNME -> SAH + PNDME');
model = addReaction(model,'R145', 'SAM + PNDME -> SAH + PC');
%model = addReaction(model,'R146
model = addReaction(model,'R147', 'G3PC -> CHO + GL3P');
model = addReaction(model,'R148', 'G3PE -> ETHA + GL3P');
model = addReaction(model,'R149', 'GL3P + ACYCOA <=> AGL3P + COA');
model = addReaction(model,'R150', 'AGL3P + ACYCOA -> PA + COA');
model = addReaction(model,'R151', 'DGLYCERATE + NADPH  + H <=> GLAL +
NADP');
model = addReaction(model,'R152', 'UDPG + ACSPG -> UDP + GLUCM');
model = addReaction(model,'R153', 'bGALbGLCM -> GLUCM + GLAC');
model = addReaction(model,'R154', 'R5P + ATP <=> PRPP + AMP');
model = addReaction(model,'R155', 'PRPP + GLN -> PPI + GLU + PRAM');
model = addReaction(model,'R156', 'AD + PRPP -> PPI + AMP');
model = addReaction(model,'R157', 'PRAM + ATP + GLY <=> ADP + PI +
GAR');
model = addReaction(model,'R158', 'GAR + FTHF -> THF + FGAR');
model = addReaction(model,'R159', 'FGAR + ATP + GLN -> GLU + ADP + PI +
FGAM');
model = addReaction(model,'R160', 'FGAM + ATP -> ADP + PI + AIR');
model = addReaction(model,'R161', 'AIR + CO2 + ATP <=> NCAIR + ADP +
PI');
model = addReaction(model,'R162', 'NCAIR <=> CAIR');
model = addReaction(model,'R163', 'CAIR + ATP + ASP <=> ADP + PI +
SAICAR');
model = addReaction(model,'R164', 'SAICAR <=> FUM + AICAR');
```

```
model = addReaction(model,'R165', 'AICAR + FTHF <=> THF + PRFICA');
model = addReaction(model,'R166', 'PRFICA <=> IMP');
model = addReaction(model,'R167', 'GMP + ATP <=> GDP + ADP');
model = addReaction(model,'R168', 'GDP + RTHIO -> DGDP + OTHIO');
model = addReaction(model,'R169', 'GTP + RTHIO -> DGTP + OTHIO');
model = addReaction(model,'R170', 'DGMP + ATP <=> DGDP + ADP');
model = addReaction(model,'R171', 'DGTP -> DG + 3 PI');
model = addReaction(model,'R172', 'DGMP <=> DG + PI');
model = addReaction(model,'R173', 'GMP -> PI + GSN');
model = addReaction(model,'R174', 'GN + PRPP -> PPI + GMP');
model = addReaction(model,'R175', 'ATP + XMP + GLN -> AMP + PPI + GMP +
GLU');
model = addReaction(model,'R176', 'XMP -> PI + XTSN');
model = addReaction(model,'R177', 'XAN + PRPP -> XMP + PPI');
model = addReaction(model,'R178', 'GN -> XAN + NH3');
model = addReaction(model,'R179', 'IMP + NAD -> NADH + XMP + H');
model = addReaction(model,'R180', 'IMP -> PI + INS');
model = addReaction(model,'R181', 'GTP + IMP + ASP <=> GDP + PI +
ASUC');
model = addReaction(model,'R182', 'ASUC <=> FUM + AMP');
model = addReaction(model,'R183', 'ADN -> INS + NH3');
model = addReaction(model,'R184', 'AMP -> PI + ADN');
model = addReaction(model,'R185', 'AD + R1P <=> PI + ADN');
model = addReaction(model,'R186', 'ATP + AMP <=> 2 ADP');
model = addReaction(model,'R187', 'ATP + RTHIO -> DATP + OTHIO');
model = addReaction(model,'R188', 'ADP + RTHIO -> DADP + OTHIO');
model = addReaction(model,'R189', 'DAMP + ATP <=> ADP + DADP');
model = addReaction(model,'R190', 'DA -> DIN + NH3');
model = addReaction(model,'R191', 'DAMP <=> DA + PI');
model = addReaction(model,'R192', 'IMP + GTP + ASP -> GDP + PI +
ASUC');
model = addReaction(model,'R193', 'XMP + ATP + GLN -> GLU + AMP + PPI +
GMP');
model = addReaction(model,'R194', 'ARIB -> AMP + R5P');
model = addReaction(model,'R195', 'ITP -> IMP + PPI');
model = addReaction(model,'R196', 'GTP -> GMP + PPI');
model = addReaction(model,'R197', 'ppGpp <=> GDP + PPI');
model = addReaction(model,'R198', 'pppGpp <=> ppGpp + PI');
model = addReaction(model,'R199', 'GTP + PYR <=> GDP + PEP');
model = addReaction(model,'R200', 'DGDP + PEP -> DGTP + PYR');
model = addReaction(model,'R201', 'DGTP -> DGMP + PPI');
model = addReaction(model,'R202', 'ATP + XMP + NH3 -> AMP + PPI +
GMP');
model = addReaction(model,'R203', 'XTP -> XMP + PPI');
model = addReaction(model,'R204', 'DATP + PYR <=> DADP + PEP');
model = addReaction(model,'R205', 'CAASP <=> DOROA');
model = addReaction(model,'R206', 'CAP + ASP -> CAASP + PI');
model = addReaction(model,'R207', '2 ATP + GLN + CO2 -> 2 ADP + PI +
GLU + CAP');    % 2 missing before ATP
model = addReaction(model,'R208', 'UTP + RTHIO -> OTHIO + DUTP');
model = addReaction(model,'R209', 'DCDP + ATP <=> DCTP + ADP');
model = addReaction(model,'R210', 'DOROA + Q <=> QH2 + OROA');
model = addReaction(model,'R211', 'OROA + PRPP <=> PPI + OMP');
model = addReaction(model,'R212', 'OMP -> CO2 + UMP');
model = addReaction(model,'R213', 'UMP + ATP <=> ADP + UDP');
```

```
model = addReaction(model,'R214', 'UDP + ATP <=> UTP + ADP');
model = addReaction(model,'R215', 'CDP + ATP <=> CTP + ADP');
model = addReaction(model,'R216', 'UTP + GLN + ATP -> GLU + CTP + ADP +
PI');
model = addReaction(model,'R217', 'CMP + ATP <=> ADP + CDP');
model = addReaction(model,'R218', 'CTP + RTHIO -> DCTP + OTHIO');
model = addReaction(model,'R219', 'CDP + RTHIO -> DCDP + OTHIO');
model = addReaction(model,'R220', 'DCMP + ATP <=> ADP + DCDP');
model = addReaction(model,'R221', 'DUTP -> PPI + DUMP');
model = addReaction(model,'R222', 'DUMP + ATP <=> DUDP + ADP');
model = addReaction(model,'R223', 'UDP + RTHIO -> DUDP + OTHIO');
model = addReaction(model,'R224', 'DUMP + METTHF -> DHF + DTMP');
model = addReaction(model,'R225', 'DTMP + ATP <=> ADP + DTDP');
model = addReaction(model,'R226', 'DTDP + ATP <=> DTTP + ADP');
model = addReaction(model,'R227', 'UMP <=> PI + URI');
model = addReaction(model,'R228', 'CYTD -> URI + NH3');
model = addReaction(model,'R229', 'CMP <=> CYTD + PI');
model = addReaction(model,'R230', 'DCMP <=> DC + PI');
model = addReaction(model,'R231', 'DC -> NH3 + DU');
model = addReaction(model,'R232', 'DU + ATP -> DUMP + ADP');
model = addReaction(model,'R233', 'DTMP -> DT + PI');
model = addReaction(model,'R234', 'DT + ATP -> ADP + DTMP');
model = addReaction(model,'R235', 'OTHIO + NADPH  + H -> RTHIO +
NADP');
model = addReaction(model,'R236', 'CTP -> UTP + NH3');
model = addReaction(model,'R237', 'ATP + UTP + NH3 -> ADP + PI + CTP');
model = addReaction(model,'R238', 'UTP -> UMP + PPI');
model = addReaction(model,'R239', 'DCTP -> DUTP + NH3');
model = addReaction(model,'R240', 'ASP + O2 -> OA + NH3 + H2O2');
model = addReaction(model,'R241', 'ASP + AKG <=> OA + GLU');
model = addReaction(model,'R242', 'ASP -> ALA + CO2');
model = addReaction(model,'R243', 'ASP + ATP + NH3 -> ASN + AMP +
PPI');
model = addReaction(model,'R244', 'ASP -> DASP');
model = addReaction(model,'R245', 'PYR + GLU <=> AKG + ALA');
model = addReaction(model,'R246', 'AKG + NH3 + NADPH  + H <=> GLU +
NADP');
model = addReaction(model,'R247', 'ADP + 3PG <=> ATP + DGLYCERATE');
model = addReaction(model,'R248', '3PG + NAD -> PHP + NADH + H');
model = addReaction(model,'R249', 'PHP + GLU -> 3PSER + AKG');
model = addReaction(model,'R250', '3PSER -> SER + PI');
model = addReaction(model,'R251', 'SER <=> PYR + NH3');
model = addReaction(model,'R252', 'THF + SER -> METTHF + GLY');
model = addReaction(model,'R253', 'SUCCOA + GLY -> ALAV + COA + CO2');
model = addReaction(model,'R254', 'THR <=> GLY + ACAL');
model = addReaction(model,'R255', 'PHSER -> THR + PI');
model = addReaction(model,'R256', 'ATP + HSER -> ADP + PHSER');
model = addReaction(model,'R257', 'HSER + NAD <=> ASPSA + NADH + H');
model = addReaction(model,'R258', 'HSER + NADP <=> ASPSA + NADPH + H');
model = addReaction(model,'R259', 'BASP + NADPH  + H -> ASPSA + PI +
NADP');
model = addReaction(model,'R260', 'ATP + ASP -> ADP + BASP');
model = addReaction(model,'R261', 'DGLY + NAD <=> HPYR + NADH + H');
model = addReaction(model,'R262', 'DGLY + NADP <=> HPYR + NADPH + H');
model = addReaction(model,'R263', 'ATHR <=> GLY + ETH');
```

```
model = addReaction(model,'R264', 'ACCOA + SER <=> COA + ASER');
model = addReaction(model,'R265', 'ASER + H2S -> CYS + AC');
model = addReaction(model,'R266', 'H2S + PYR + NH3 -> CYS');
model = addReaction(model,'R267', 'SAH <=> HCYS + ADN');
model = addReaction(model,'R268', 'MTHF + HCYS <=> THF + MET');
model = addReaction(model,'R269', 'ATP + MET -> PI + PPI + SAM');
model = addReaction(model,'R270', 'SAM + H <=> DSAM + CO2');
model = addReaction(model,'R271', 'PTRC + DSAM -> SPMD + 5MTA + H');
model = addReaction(model,'R272', '5MTA -> AD + 5MTR');
model = addReaction(model,'R273', '5MTR + ATP -> 5MTRP + ADP');
model = addReaction(model,'R274', '5MTRP <=> 5MTR1P');
model = addReaction(model,'R275', '5MTR1P -> DKMPP');
model = addReaction(model,'R276', 'DKMPP -> FORT + KMB');
model = addReaction(model,'R277', 'KMB + GLN -> GLU + MET');
model = addReaction(model,'R278', 'SER -> 2AA');
model = addReaction(model,'R279', 'ASER + HO3S2 + RTHIO -> CYS + H2SO3
+ OTHIO + AC');
model = addReaction(model,'R280', 'CYST -> PYR + NH3 + TCYS');
model = addReaction(model,'R281', 'CYSTEATE + AKG <=> 3SPYR + GLU');
model = addReaction(model,'R282', 'CYS + AKG <=> MPYR + GLU');
model = addReaction(model,'R283', '3SLALA + AKG -> 3SFPYR + GLU');
model = addReaction(model,'R284', '3SLALA -> ALA + SO2');
model = addReaction(model,'R285', 'MTGLU + HCYS -> TGLU + MET');
model = addReaction(model,'R286', 'MET -> MTE + NH3 + OBUT');
model = addReaction(model,'R287', 'KMB + GLU -> MET + AKG');
model = addReaction(model,'R288', 'THR -> OBUT + NH3');
model = addReaction(model,'R289', 'OBUT + HTPP -> TPP + ABUT');
model = addReaction(model,'R290', 'ABUT <=> HMOPENT');
model = addReaction(model,'R291', 'DHMB -> MOBA');
model = addReaction(model,'R292', 'HTPP + PYR -> ACLAC + TPP');
model = addReaction(model,'R293', 'ACCOA + MOBA -> IPPMAL + COA');
model = addReaction(model,'R294', 'MOPENA + GLU -> ILE + AKG');
model = addReaction(model,'R295', 'MOBA + GLU -> VAL + AKG');
model = addReaction(model,'R296', 'IPPMAL + NAD -> OICAP + NADH + H');
model = addReaction(model,'R297', 'OICAP + GLU <=> LEU + AKG');
model = addReaction(model,'R298', 'ACCOA + MOBA -> IPPMAL + COA');
model = addReaction(model,'R299', 'E3MM + NAD -> OBUT + CO2 + NADH +
H');
model = addReaction(model,'R300', '2MM -> E3MM');
model = addReaction(model,'R301', 'R2MM -> 2MM');
model = addReaction(model,'R302', 'HTPP + PYR -> ACLAC + TPP');
model = addReaction(model,'R303', 'OBUT + HTPP -> TPP + ABUT');
model = addReaction(model,'R304', 'ABUT <=> HMOPENT');
model = addReaction(model,'R305', 'ACLAC <=> HMOBA');
model = addReaction(model,'R306', 'HMOPENT + NADPH + H <=> DHMP +
NADP');
model = addReaction(model,'R307', 'HMOBA + NADPH + H <=> DHMB + NADP');
model = addReaction(model,'R308', 'DHMP -> MOPENA');
model = addReaction(model,'R309', 'DHMB -> MOBA');
model = addReaction(model,'R310', 'MOBA + GLU -> VAL + AKG');
model = addReaction(model,'R311', 'IPPMAL <=> IPPMALE');
model = addReaction(model,'R312', 'ASPSA + PYR -> D23PIC');
model = addReaction(model,'R313', 'D23PIC + NADPH + H -> PIP26DX +
NADP');
model = addReaction(model,'R314', 'SUCCOA + PIP26DX -> COA + NS2A6O');
```

```
model = addReaction(model,'R315', 'NS26DP + AKG <=> NS2A6O + GLU');
model = addReaction(model,'R316', 'NS26DP -> SUCC + D26PIM');
model = addReaction(model,'R317', 'D26PIM <=> MDAP');
model = addReaction(model,'R318', 'MDAP -> LYS + CO2');
model = addReaction(model,'R319', 'UDPNAMAG + ATP + MDAP -> UNAGD + ADP
+ PI');
model = addReaction(model,'R320', 'UNAGD + ATP + AA -> UNAGDA + ADP +
PI');
model = addReaction(model,'R321', 'ACCOA + AKG -> HCT + COA');
model = addReaction(model,'R322', 'ATP + CITR + ASP <=> AMP + PPI +
ARGSUCC');
model = addReaction(model,'R323', 'ARGSUCC <=> FUM + ARG');
model = addReaction(model,'R324', 'D23PIC + NADH + H -> PIP26DX +
NAD');
model = addReaction(model,'R325', 'ARG -> ORN + UREA');
model = addReaction(model,'R326', 'NAGLUSAL + GLU <=> AKG + NAARON');
model = addReaction(model,'R327', 'NAGLUYP + NADPH + H <=> NADP + PI +
NAGLUSAL');
model = addReaction(model,'R328', 'NAGLU + ATP -> ADP + NAGLUYP');
model = addReaction(model,'R329', 'GLU + ACCOA -> COA + NAGLU');
model = addReaction(model,'R330', 'ATP + GLU -> ADP + GLUP');
model = addReaction(model,'R331', 'GLUP + NADPH + H -> GLUGSAL + PI +
NADP');
model = addReaction(model,'R332', 'ORN -> PTRC + CO2');
model = addReaction(model,'R333', 'PRO + NADP <=> PYCA + NADPH + H');
model = addReaction(model,'R334', 'SUCCOA + ARG -> COA + SUCCARG');
model = addReaction(model,'R335', 'ORN <=> PRO + NH3');
model = addReaction(model,'R336', 'PRO + NAD <=> PYCA + NADH + H');
model = addReaction(model,'R337', 'SUCCGLU5SAL + NAD -> SUCCGLU + NADH
+ H');
model = addReaction(model,'R338', 'PYRRHCAR + NADH + H -> HPRO + NAD');
model = addReaction(model,'R339', 'PYRRHCAR + NADPH + H -> HPRO +
NADP');
model = addReaction(model,'R340', 'E4HGLU + AKG -> HYDROXYAKG + GLU');
model = addReaction(model,'R341', 'AGMAT -> NCPTRC + NH3');
model = addReaction(model,'R342', 'SUCCARG -> SUCCORN + CO2 + 2 NH3');
model = addReaction(model,'R343', 'NCPTRC -> PTRC + CO2 + NH3');
model = addReaction(model,'R344', 'CAP + ORN <=> CITR + PI');
model = addReaction(model,'R345', 'ORN + AKG <=> PYCA + GLU');
model = addReaction(model,'R346', 'NAARON + GLU <=> ORN + NAGLU');
model = addReaction(model,'R347', 'ATP + PRPP <=> PRBATP + PPI');
model = addReaction(model,'R348', 'PRBATP -> PRBAMP + PPI');
model = addReaction(model,'R349', 'PRBAMP -> PRFP');
model = addReaction(model,'R350', 'PRFP -> PRLP');
model = addReaction(model,'R351', 'PRLP + GLN -> AICAR + GLU + DIMGP');
model = addReaction(model,'R352', 'DIMGP -> IMACP');
model = addReaction(model,'R353', 'IMACP + GLU -> HISOLP + AKG');
model = addReaction(model,'R354', 'HISOLP -> HISOL + PI');
model = addReaction(model,'R355', 'HISOL + NAD -> HISAL + NADH + H');
model = addReaction(model,'R356', 'HISAL + NAD -> HIS + NADH + H');
model = addReaction(model,'R357', 'DHPEG + NAD <=> DHMA + NADH + H');
model = addReaction(model,'R358', 'IDPYR -> IDACAL + CO2');
model = addReaction(model,'R359', 'IDACTN -> IAC + NH3');
model = addReaction(model,'R360', 'PEP + E4P -> 3DDAH7P + PI');
model = addReaction(model,'R361', '3DDAH7P -> DOT + PI');
```

```
model = addReaction(model,'R362', 'DOT <=> DHSK');
model = addReaction(model,'R363', 'SME + NADP <=> DHSK + NADPH + H');
model = addReaction(model,'R364', 'ATP + SME -> ADP + SME5P');
model = addReaction(model,'R365', 'PEP + SME5P <=> PI + 3PSME');
model = addReaction(model,'R366', '3PSME -> CHOR + PI');
model = addReaction(model,'R367', 'INDOLE + T3P1 <=> IGP');
model = addReaction(model,'R368', 'SER + INDOLE -> TRP');
model = addReaction(model,'R369', 'SER + IGP <=> TRP + T3P1');
model = addReaction(model,'R370', 'CPAD5P -> CO2 + IGP');
model = addReaction(model,'R371', 'NPRAN -> CPAD5P');
model = addReaction(model,'R372', 'AN + PRPP -> PPI + NPRAN');
model = addReaction(model,'R373', 'CHOR + NH3 -> AN + PYR');
model = addReaction(model,'R374', 'CHOR + GLN -> AN + PYR + GLU');
model = addReaction(model,'R375', 'CHOR <=> PHEN');
model = addReaction(model,'R376', 'PHEN <=> PHPYR + CO2');
model = addReaction(model,'R377', 'PHE + AKG <=> PHPYR + GLU');
model = addReaction(model,'R378', 'TYR + AKG <=> HPHPYR + GLU');
model = addReaction(model,'R379', 'ASP + PHEN -> OA + AG');
model = addReaction(model,'R380', 'AG -> PHE + CO2');
model = addReaction(model,'R381', 'AG + NAD -> TYR + CO2 + NADH + H');
model = addReaction(model,'R382', 'AG + NADP -> TYR + CO2 + NADPH +
H');
model = addReaction(model,'R383', 'SLLCT -> SHCYS + NH3 + PYR');
model = addReaction(model,'R384', 'ADSHCYS -> ADN + SHCYS');
model = addReaction(model,'R385', 'ATP + SMET -> PI + PPI + ADSMET');
model = addReaction(model,'R386', 'SMET -> MTSEL + NH3 + OBUT');
model = addReaction(model,'R387', 'ASER + SELD -> SCYS + AC');
model = addReaction(model,'R388', 'ATP + SELNT <=> PPI + ADSELNT');
model = addReaction(model,'R389', 'ATP + ADSELNT -> ADP + 3PADSELNT');
model = addReaction(model,'R390', 'APRCN -> ALA + NH3');
model = addReaction(model,'R391', 'ACYABUT -> GLU + NH3');
model = addReaction(model,'R392', 'CYAALA + GLU -> GLUCYALA');
model = addReaction(model,'R393', 'APRCN + GLU -> GLUAPRCN');
model = addReaction(model,'R394', 'ASN -> ASP + NH3');
model = addReaction(model,'R395', 'GLU <=> DGLU');
model = addReaction(model,'R396', 'UDPNAMA + DGLU + ATP -> UDPNAMAG +
ADP + PI');
model = addReaction(model,'R397', 'UDPNAM + ALA + ATP -> ADP + PI +
UDPNAMA');
model = addReaction(model,'R398', 'ALA <=> DALA');
model = addReaction(model,'R399', 'ATP + 2 DALA -> ADP + PI + AA');
model = addReaction(model,'R400', 'ATP + GLU + CYS -> ADP + PI + GC');
model = addReaction(model,'R401', 'GC -> CYS + GLY');
model = addReaction(model,'R402', 'ATP + GC + GLY -> ADP + PI + RGT');
model = addReaction(model,'R403', 'OGT + NADPH + H <=> 2 RGT + NADP');
model = addReaction(model,'R404', 'CYSGLY -> CYS + GLY');
model = addReaction(model,'R405', 'RGT -> CYSGLY + GLU');
model = addReaction(model,'R406', 'ICIT + NADP -> AKG + CO2 + NADPH +
H'); %not added
model = addReaction(model,'R407', 'RX + RGT -> HAL + RSGSH');
model = addReaction(model,'R408', 'RSGSH -> RSCYSGLY + GLU');
model = addReaction(model,'R409', 'RSCYSGLY -> RSCYS + GLY');
model = addReaction(model,'R410', 'UNAGDA + UDCPP -> UMP + PI +
UAGMDA');
model = addReaction(model,'R411', 'UAGMDA + UDPNAG -> UDP + UAAGMDA');
```

```
model = addReaction(model,'R412', 'UAAGMDA -> UDCPDP + PEPTIDO');
model = addReaction(model,'R413', 'UDCPDP -> UDCPP + PI');
model = addReaction(model,'R414', 'PPEPTIDO + DALA -> PEPTIDO +
DALAxt');
model = addReaction(model,'R415', 'AIR -> AHM');
model = addReaction(model,'R416', 'THZP + AHMPP + H -> THMP + PPI');
model = addReaction(model,'R417', 'THMP + ATP <=> TPP + ADP');
model = addReaction(model,'R418', 'ATP + AMPMPM -> ADP + AHMPP');
model = addReaction(model,'R419', 'C15815 + DX5P + TYR -> THZP');
model = addReaction(model,'R420', 'C15815 + TYR + IMGLY -> THZP');
model = addReaction(model,'R421', 'THMPP -> THMP + PI');
model = addReaction(model,'R422', 'GTP -> FORT + D6RP5P + PPI');
model = addReaction(model,'R423', 'D6RP5P -> A6RP5P + NH3');
model = addReaction(model,'R424', 'A6RP5P + NADPH + H -> A6RP5P2 +
NADP');
model = addReaction(model,'R425', 'A6RP5P2 -> A6RP + PI');
model = addReaction(model,'R426', 'RL5P -> DB4P + FORT');
model = addReaction(model,'R427', 'DB4P + A6RP -> D8RL + PI');
model = addReaction(model,'R428', '2 D8RL -> RIBFLV + A6RP');
model = addReaction(model,'R429', 'RIBFLV + ATP -> ADP + FMN');
model = addReaction(model,'R430', 'FMN + ATP -> PPI + FAD');
model = addReaction(model,'R431', 'FMN -> RIBFLV + PI');
model = addReaction(model,'R432', 'PL + O2 + NH3 <=> PDLA + H2O2');
model = addReaction(model,'R433', 'PDLA5P + O2 <=> PL5P + H2O2 + NH3');
model = addReaction(model,'R434', 'PYRDX + O2 <=> PL + H2O2');
model = addReaction(model,'R435', 'P5P + O2 <=> PL5P + H2O2');
model = addReaction(model,'R436', 'OHB + GLU <=> PHT + AKG');
model = addReaction(model,'R437', 'PHT -> 4HLT + PI');
model = addReaction(model,'R438', 'AOPP + DX5P -> P5P + PI');
model = addReaction(model,'R439', 'PHT + NAD -> AOPOB + NADH + H');
model = addReaction(model,'R440', 'ATP + NAD -> ADP + NADP');
model = addReaction(model,'R441', 'NAMN + ATP <=> PPI + NAAD');
model = addReaction(model,'R442', 'NMN + ATP <=> PPI + NAD');
model = addReaction(model,'R443', 'ATP + NAAD + NH3 -> AMP + PPI +
NAD');
model = addReaction(model,'R444', 'QA + PRPP -> NAMN + CO2 + PPI');
model = addReaction(model,'R445', 'ISUCC + T3P2 -> PI + QA');
model = addReaction(model,'R446', 'NAD + ATP -> ADP + NADP');
model = addReaction(model,'R447', 'NMN -> PI + RNAM');
model = addReaction(model,'R448', 'NAMN -> PI + NAMNS');
model = addReaction(model,'R449', 'ASP + O2 -> ISUCC + H2O2'); %check
model = addReaction(model,'R450', 'ACLAC + NADPH + H <=> MOBA + NADP');
model = addReaction(model,'R451', 'MOBA + METTHF -> AKP + THF');
model = addReaction(model,'R452', 'AKP + NADPH + H -> NADP + PANT');
model = addReaction(model,'R453', 'PANT + bALA + ATP -> AMP + PPI +
PNTO');
model = addReaction(model,'R454', 'ASP -> CO2 + bALA');
model = addReaction(model,'R455', 'PNTO + ATP -> ADP + 4PPNTO');
model = addReaction(model,'R456', '4PPNTO + CTP + CYS -> CMP + PPI +
4PPNCYS');
model = addReaction(model,'R457', '4PPNCYS -> CO2 + 4PPNTE');
model = addReaction(model,'R458', 'COA -> PAP + ACP');
model = addReaction(model,'R459', '4PPNTE + ATP -> PPI + DPCOA');
model = addReaction(model,'R460', 'DPCOA + ATP -> ADP + COA');
```

```
model = addReaction(model,'R461', 'ATP + 4PPNTO + CYS -> AMP + PPI +
4PPNCYS');
model = addReaction(model,'R462', 'ATP + PNCYS -> ADP + 4PPNCYS');
model = addReaction(model,'R463', 'ATP + PTT -> ADP + 4PPNTE');
model = addReaction(model,'R464', 'ALA + CHCOA <=> CO2 + COA + AONA');
model = addReaction(model,'R465', 'SAM + AONA <=> SAMOB + DANNA');
model = addReaction(model,'R466', 'CO2 + DANNA + ATP <=> DTB + PI +
ADP');
model = addReaction(model,'R467', 'DTB + S + 2 SAM -> BT + 2 MET + 2
DA');
model = addReaction(model,'R468', 'ATP + BT -> PPI + B5AMP');
model = addReaction(model,'R469', 'AHTD -> DHP + 3 PI');
model = addReaction(model,'R470', 'DHP -> AHHMP + GLAL');
model = addReaction(model,'R471', 'AHHMP + ATP -> AMP + AHHMD');
model = addReaction(model,'R472', 'AHHMP + PABA -> DHPT');
model = addReaction(model,'R473', 'AHHMD + PABA -> PPI + DHPT');
model = addReaction(model,'R474', 'DHPT + ATP + GLU -> ADP + PI +
DHF');
model = addReaction(model,'R475', 'CHOR + GLN -> ADCHOR + GLU');
model = addReaction(model,'R476', 'DHF + NADPH + H <=> NADP + THF');
model = addReaction(model,'R477', 'ADCHOR -> PYR + PABA');
model = addReaction(model,'R478', 'GTP -> FAPNTP');
model = addReaction(model,'R479', 'FAPNTP -> DAPMNTP + FORT');
model = addReaction(model,'R480', 'DAPMNTP -> AHTD');
model = addReaction(model,'R481', 'DATPTHOPAOPM -> AHTD');
model = addReaction(model,'R482', 'AHTD -> PYTHP + PPPI');
model = addReaction(model,'R483', 'DHF + NAD <=> FL + NADH + H');
model = addReaction(model,'R484', 'DHF + NADP <=> FL + NADPH + H');
model = addReaction(model,'R485', 'FL + 2 NADH + 2 H -> THF + 2 NAD');
model = addReaction(model,'R486', 'FL + 2 NADPH + 2 H -> THF + 2
NADP');
model = addReaction(model,'R487', 'FTHF <=> METHF');
model = addReaction(model,'R488', 'METHF + NADPH + H <=> METTHF +
NADP');
model = addReaction(model,'R489', 'THF + FORT + ATP -> ADP + PI +
FTHF');
model = addReaction(model,'R490', 'DHF + NADH + H -> THF + NAD');
model = addReaction(model,'R491', 'GAR + METHF -> FGAR + THF');
model = addReaction(model,'R492', 'ATP + FTHF -> ADP + PI + METHF');
model = addReaction(model,'R493', '4 PBG -> HMB + 4 NH3');
model = addReaction(model,'R494', 'HMB -> UPRG');
model = addReaction(model,'R495', '2 SAM + UPRG -> 2 SAH + PC2');
model = addReaction(model,'R496', 'UPRG -> 4 CO2 + CPP');
model = addReaction(model,'R497', 'O2 + CPP + 2 H -> 2 CO2 + PPHG');
model = addReaction(model,'R498', 'PPIX + 2 H -> PTH + Fe');% ADDED FE
model = addReaction(model,'R499', '2 ALAV -> PBG');
model = addReaction(model,'R500', 'SHCL + Fe -> SHEME');
model = addReaction(model,'R501', 'UPRG -> CPP + 4 CO2');
model = addReaction(model,'R502', 'PC2 + NAD -> SHCL + NADH + H');
model = addReaction(model,'R503', 'CPP + 2 SAM -> PPHG + 2 CO2 + 2 MET
+ 2 DA');
model = addReaction(model,'R504', '4HBZ + OPP -> O4HBZ + PPI');
model = addReaction(model,'R505', 'O4HBZ -> CO2 + 2OPPP');
model = addReaction(model,'R506', '2OPPP + O2 + NADPH + H -> 2O6H +
NADP');
```

```
model = addReaction(model,'R507', '2O6H + SAM -> 2OPMP + SAH');
model = addReaction(model,'R508', '2OPMP + O2 + NADPH + H -> 2OPMB +
NADP');
model = addReaction(model,'R509', '2OPMB + SAM -> 2OPMMB + SAH');
model = addReaction(model,'R510', '2OPMMB + O2 + NADPH + H -> 2OMHMB +
NADP');
model = addReaction(model,'R511', '2OMHMB + SAM -> QH2 + SAH');
model = addReaction(model,'R512', 'PPPP + 4HB -> H3PPBZ + PPI');
model = addReaction(model,'R513', 'PPPH + O2 + NADPH + H -> PP6HPH +
NADP');
model = addReaction(model,'R514', 'PP6HPH + SAM -> PP6MPH + SAH');
model = addReaction(model,'R515', 'PP6MPH + O2 -> PP6M14BQ'); % CHECK
model = addReaction(model,'R516', 'PP6M14BQ + SAM -> PP3M6M14BQ +
SAH');
model = addReaction(model,'R517', 'PP3M6M14BQ + O2 + NADPH + H ->
PP3M5H6M14BQ + NADP'); %NNEDS MORE H
model = addReaction(model,'R518', 'PP3M5H6M14BQ + SAM -> Q + SAH');
model = addReaction(model,'R519', 'DMQ + SAM -> MQ + SAH');
model = addReaction(model,'R520', '2P14NQ + SAM -> PQ + SAH');
model = addReaction(model,'R521', 'ICHOR -> DHDHBZ + PYR');
model = addReaction(model,'R522', 'PYR + T3P1 -> DX5P + CO2');
model = addReaction(model,'R523', 'DX5P + NADPH + H -> MDE4P + NADP');
model = addReaction(model,'R524', 'MDE4P + CTP -> CDPMDE + PPI');
model = addReaction(model,'R525', 'CDPMDE + ATP -> 2PCDPMDE + ADP');
model = addReaction(model,'R526', '2PCDPMDE -> MDECPP + CMP');
model = addReaction(model,'R527', 'MDECPP + NADH + H -> NAD + HMB4PP');
model = addReaction(model,'R528', 'HMB4PP + NADH + H -> NAD + IPPP');
model = addReaction(model,'R529', 'IPPP -> DMPP');
model = addReaction(model,'R530', 'DMPP + IPPP -> GPP + PPI');
model = addReaction(model,'R531', 'GPP + IPPP -> FPP + PPI');
model = addReaction(model,'R532', '2 FPP + NADPH + H -> SQL + 2 PPI +
NADP');
model = addReaction(model,'R533', 'SQL -> 5 HOPENE');
model = addReaction(model,'R534', 'SQL -> HOPANOL');
model = addReaction(model,'R535', 'HOPENE -> THBH');
model = addReaction(model,'R536', 'THBH + UDPNAG -> THBHGA + THBHET');
model = addReaction(model,'R537', 'THBHGA <=> THBHET');
model = addReaction(model,'R538', 'MDECPP + 2 RFD + 2 H -> HMB4PP + 2
OFD');
model = addReaction(model,'R539', 'HMB4PP + NADPH + H -> NADP + IPPP');
model = addReaction(model,'R540', 'HMB4PP + NADH + H -> NAD + DMPP');
model = addReaction(model,'R541', 'HMB4PP + NADPH + H -> NADP + DMPP');
model = addReaction(model,'R542', 'FPP + IPP -> GGPP + PPI');
model = addReaction(model,'R543', 'GGPP + 7 IPP -> UDCPP + 7 PPI');
model = addReaction(model,'R544', 'HEPPP + IPP -> OPPPI + PPI');
model = addReaction(model,'R545', 'G6P -> I3P');
model = addReaction(model,'R546', 'PHEN + NAD <=> HPHPYR + CO2 +
NADH');
model = addReaction(model,'R547', 'DCLCMBO -> DCLOHE');
model = addReaction(model,'R548', 'CMBO -> 2MAC');
model = addReaction(model,'R549', 'NPHP -> NPH + PI');
model = addReaction(model,'R550', 'CLPOL + NAD -> CLAALD + NADH + H');
model = addReaction(model,'R551', 'VAN + O2 + NADH + H -> DHBZ + NAD +
FALD');
model = addReaction(model,'R552', 'SULLAC -> HSO3 + 2MAC');
```

```
model = addReaction(model,'R553', 'HC2H + RFD + ATP + H -> C2H4 + OFD +
ADP + PI');
model = addReaction(model,'R554', 'EO + COA + NAD -> ACCOA + NADH +
H');
model = addReaction(model,'R555', 'CLCMBO -> CLMAC');
model = addReaction(model,'R556', 'PAMN -> ACAC');
model = addReaction(model,'R557', 'BZNIT -> BZ + NH3');
model = addReaction(model,'R558', 'ODHFAC -> OAP');
model = addReaction(model,'R559', 'HMNAPTH + NAD -> NAPTHAH + NADH');
model = addReaction(model,'R560', 'NAPTHM + NAD -> NAPTHAH + NADH');
model = addReaction(model,'R561', 'FMUCLAC -> 2MAC + HFA');
model = addReaction(model,'R562', 'NH3xt + HEXT <=> NH3 + H');
model = addReaction(model,'R563', 'Kxt + HEXT <=> K + H');
model = addReaction(model,'R564', 'SLFxt + ATP -> SLF + ADP + PI + H');
%making reversible
model = addReaction(model,'R565', 'PIxt + ATP -> ADP + 2 PI + H');
%making reversible
model = addReaction(model,'R566', 'SUCCxt + HEXT <=> SUCC + H');
model = addReaction(model,'R567', 'FUMxt + HEXT <=> FUM + H');
model = addReaction(model,'R568', 'MALxt + HEXT <=> MAL + H');
model = addReaction(model,'R569', 'NAxt + H <=> NA + HEXT');
model = addReaction(model,'R570', 'CO2xt <=> CO2');
model = addReaction(model,'R571', 'ATP -> ADP + PI + H');
model = addReaction(model,'R572', 'SOBxt + H <=> SOB + HEXT');
model = addReaction(model,'R573', 'SOT + H <=> SOTxt + HEXT');
model = addReaction(model,'R574', 'AC + H <=> ACxt + HEXT');
model = addReaction(model,'R575', 'LAC + H <=> LACxt + HEXT');
model = addReaction(model,'R576', 'FORT <=> FORTxt');
model = addReaction(model,'R577', 'GLCxt -> GLC');
model = addReaction(model,'R578', 'LEVAN <=> LEVANxt');
model = addReaction(model,'R579', 'ACTN + H <=> ACTNxt + HEXT');
model = addReaction(model,'R580', 'NADxt -> NMNxt + AMPxt');
model = addReaction(model,'R581', 'NMNxt -> NMN');
model = addReaction(model,'R582', 'NMNxt -> R5P + NAM');
model = addReaction(model,'R583', 'NACxt -> NAC');
model = addReaction(model,'R584', 'ETH + H -> ETHxt + HEXT');
model = addReaction(model,'R585', 'O2xt <=> O2');
model = addReaction(model,'R586', 'SUCxt -> SUC');
model = addReaction(model,'R587', 'ACALxt + H <=> ACAL + HEXT');
model = addReaction(model,'R588', 'GLxt <=> GL');
model = addReaction(model,'R589', 'DHACTxt <=> DHACT');
model = addReaction(model,'R590', 'PNTOxt + HEXT <=> PNTO + H');
model = addReaction(model,'R591', 'FRUxt <=> FRU');
model = addReaction(model,'R592', '0.869 DATP + 0.75 DCTP + 0.869 DTTP
+ 0.75 DGTP + 4.4 ATP -> 4.4 ADP + 4.4 PI + 3.237 PPI + DNA');
model = addReaction(model,'R593', '0.667 ATP + 0.929 GTP + 0.718 CTP +
0.786 UTP -> 1.24 ADP + 1.24 PI + RNA + 3.1 PPI');   % hydrogen added
model = addReaction(model,'R594', '2.144 ALA + 0.317 ARG + 0.435 ASN +
0.435 ASP + 0.037 CYS + 0.308 GLN + 0.307 GLU + 1.903 GLY + 0.146 HIS +
0.672 ILE + 0.672 LEU + 0.447 LYS + 0.145 MET + 0.019 PHE + 0.391 PRO +
0.383 SER + 0.415 THR + 0.093 TRP + 0.122 TYR + 1.057 VAL + 44.92 ATP -
> 44.92 ADP + 44.92 PI + PROTEIN ');  % hydrogen added
model = addReaction(model,'R595', '0.118 CL + 0.655 PE + 0.054 PG +
0.141 PINSTOL + 0.25 PC -> PHOSPHOLIPID');
```

```
model = addReaction(model,'R596', 'GL3P + 0.1 C140ACP + 0.1 C160ACP +
0.02 C161ACP + 0.78 C181ACP -> AGL3P + ACP');
model = addReaction(model,'R597', 'AGL3P + 0.1 C140ACP + 0.1 C160ACP +
0.02 C161ACP + 0.78 C181ACP -> PA + ACP');
model = addReaction(model,'R598', '0.11 THBH + 0.692 THBHGA + 0.579
THBHET + 0.07 HOPANOL + 0.024 HOPENE -> HOPANOIDS');
model = addReaction(model,'R599', '1.235 GL3P + 0.408 C120ACP + 0.445
C140ACP + 0.222 C141ACP + 0.556 C160ACP + 0.593 C161ACP + 0.111 C180ACP
+ 1.112 C181ACP + 0.259 C190ACP -> TAG + 3.705 ACP + 1.235 PI');
model = addReaction(model,'R600', '0.167 NAD + 0.149 NADP + 0.145 COA +
0.01 ACP + 1.26 PTRC + 0.765 SPMD + 0.249 THF + 0.243 FMN + 0.141 FAD -
> SMALL_MOLECULES');
model = addReaction(model,'R601', '0.195 RNA + 0.027 DNA + 0.605
PROTEIN + 0.053 PHOSPHOLIPID + 0.025 PEPTIDO + 0.025 GLYCOGEN + 0.038
SMALL_MOLECULES + 16.45 ATP + 0.028 HOPANOIDS + 0.004 TAG <=> Biomass +
16.45 ADP + 16.45 PI ');     % added hydrogen

% Add Export reactions
%
model = addReaction(model,'EX_562', 'NH3xt');
model = addReaction(model,'EX_562_1', 'HEXT'); % proton export??   %del
model = addReaction(model,'EX_563', 'Kxt');
model = addReaction(model,'EX_564', 'SLFxt'); %del
model = addReaction(model,'EX_565', 'PIxt');
model = addReaction(model,'EX_566', 'SUCCxt ->');   %del
model = addReaction(model,'EX_567', 'FUMxt ->');     %del
model = addReaction(model,'EX_568', 'MALxt ->');     %del
model = addReaction(model,'EX_569', 'NAxt');
model = addReaction(model,'EX_570', 'CO2xt ->');
model = addReaction(model,'EX_572', 'SOBxt ->');
model = addReaction(model,'EX_573', 'SOTxt ->');
model = addReaction(model,'EX_574', 'ACxt ->');
model = addReaction(model,'EX_575', 'LACxt ->');
model = addReaction(model,'EX_576', 'FORTxt ->');
model = addReaction(model,'EX_577', 'GLCxt');
model = addReaction(model,'EX_578', 'LEVANxt');
model = addReaction(model,'EX_579', 'ACTNxt ->');
model = addReaction(model,'EX_580', 'NADxt');    %del
model = addReaction(model,'EX_580_1', 'AMPxt ->');  %del
model = addReaction(model,'EX_581', 'NMNxt ->');   %del % essential?!
model = addReaction(model,'EX_583', 'NACxt ->');
model = addReaction(model,'EX_584', 'ETHxt ->');
model = addReaction(model,'EX_585', 'O2xt');
model = addReaction(model,'EX_586', 'SUCxt'); %del
model = addReaction(model,'EX_587', 'ACALxt ->');
model = addReaction(model,'EX_588', 'GLxt');  % source
model = addReaction(model,'EX_589', 'DHACTxt'); %del
model = addReaction(model,'EX_590', 'PNTOxt'); %del
model = addReaction(model,'EX_591', 'FRUxt'); %del
model = addReaction(model,'EX_Biomass', 'Biomass ->');
%}
model = changeObjective(model, 'R601');

model=changeRxnBounds(model,'EX_588', 0, 'b');%Glycerol
model=changeRxnBounds(model,'EX_577', 0, 'b');%Glucose
```

```
model=changeRxnBounds(model,'EX_578', 0, 'b');%Levan
model=changeRxnBounds(model,'EX_585', 0, 'b');%Oxygen
model=changeRxnBounds(model,'EX_589', 0, 'b');%DHAC
model=changeRxnBounds(model,'EX_590', 0, 'b');%PNTO
model=changeRxnBounds(model,'EX_591', 0, 'b');%Fructose
model=changeRxnBounds(model,'EX_586', 0, 'b');%Sucrose
model=changeRxnBounds(model,'EX_580', 0, 'b');%nad
%model=changeRxnBounds(model,'R100', 0, 'b');
%model=changeRxnBounds(model,'EX_562',-3.494, 'b');%amonia
%model=changeRxnBounds(model,'R571', 1000, 'b');
%reverse testing after atp

%Glycerol Metabolism
%{
model = addReaction(model,'R602', 'GL + NAD -> DHACT + NADH + H');
model = addReaction(model,'R603', 'DHACT + ATP -> T3P2 + ADP');
model = addReaction(model,'R604', 'GL + NADH + H -> 13PDO + NAD');
model = addReaction(model,'EX_592', '13PDO');
%}
%pentose sugar metabolism
model = addReaction(model,'R602', 'XYL');
model = addReaction(model,'R603', 'XYL <=> XYLU');
model = addReaction(model,'R604', 'XYLU + ATP <=> X5P + ADP');
model = addReaction(model,'R605', 'ARA <=> RIB');
model = addReaction(model,'R606', 'RIB + ATP <=> LRL5P + ADP');
model = addReaction(model,'R607', 'LRL5P <=> X5P');
model = addReaction(model,'R608', 'ARA');
model = addReaction(model,'R609', 'S7P + T3P1 <=> E4P + F6P');

model = addReaction(model,'R097_1', 'O2 + 4 ccfe2 + 4 H -> 4 ccfe3 + 4
HEXT');% adding reaction to ensure cytochrome consumption.
model = addReaction(model,'R449_1', 'ASP + FUM -> ISUCC + SUCC + H');
model = addReaction(model,'BR52_1', 'ACCOA + NADH + H <=> ACAL + COA +
NAD');%
model = addReaction(model,'BR52_2', 'ACAL + NAD <=> AC + NADH + H');
%model = addReaction(model,'R605', 'FAD + NADH + H -> FADH2 + NAD');
model=changeRxnBounds(model,'R602', -12, 'l');
model=changeRxnBounds(model,'R602', 0, 'u');
model=changeRxnBounds(model,'R608', 0, 'b');
model=changeRxnBounds(model,'R571', 1.1, 'b');%fixing maintenance value


%Acetate removal
%model=changeRxnBounds(model,'EX_574', 0, 'b');%Acetate
model=changeRxnBounds(model,'R023', 0, 'b');
model=changeRxnBounds(model,'BR52_2', 0, 'b');
%formate removal
%model=changeRxnBounds(model,'EX_576', 0, 'b');%Formate
%model=changeRxnBounds(model,'R075', 0, 'b');% removing stopped growth

%ETHANOL REMOVAL
%model=changeRxnBounds(model,'EX_584', 0, 'b');%Ethanol
model=changeRxnBounds(model,'R019', 0, 'b');%Ethanol
%model=changeRxnBounds(model,'R263', 0, 'b');%Ethanol%not needed
```

```
%Acetaldehyde removal
%%need to find out if export exists
%model=changeRxnBounds(model,'EX_587', 0, 'b');%Acetaldehyde
model=changeRxnBounds(model,'BR52_1', 0, 'b');%removing didnt do much
change but made the solution space near the optimum case sharper.
model=changeRxnBounds(model,'R018', 0, 'b');


%succinate removal
%model=changeRxnBounds(model,'EX_566', 0, 'b');%Succinate
model=changeRxnBounds(model,'R027', 0, 'b');%Succinate
model=changeRxnBounds(model,'R102', 0, 'b');%Succinate
%acetoin removal
%model=changeRxnBounds(model,'EX_579', 0, 'b');%Acetoin
model=changeRxnBounds(model,'R090', 0, 'b');%Acetoin

%Lactate removal-presence shows growth and more alkane
%model=changeRxnBounds(model,'EX_575', 0, 'b');%Lactate
model=changeRxnBounds(model,'R073', 0, 'b');%Lactate

%malate removal
%model=changeRxnBounds(model,'EX_568', 0, 'b');%Malate
%needed else succ is imp
model=changeRxnBounds(model,'R076', 0, 'b');%Malate
model=changeRxnBounds(model,'R026', 0, 'b');%Malate

%Fumarate removal
%model=changeRxnBounds(model,'EX_567', 0, 'b');%Fumurate
%model=changeRxnBounds(model,'R164', 0, 'b');%Fumurate
%model=changeRxnBounds(model,'R182', 0, 'b');%Fumurate
%model=changeRxnBounds(model,'R323', 0, 'b');%Fumurate


%removing cycles?
%not required to remove
%{
model=changeRxnBounds(model,'R546', 0, 'b');%
model=changeRxnBounds(model,'R258', 0, 'b');%
model=changeRxnBounds(model,'R476', 0, 'b');%
model=changeRxnBounds(model,'R484', 0, 'b');%
model=changeRxnBounds(model,'R262', 0, 'b');%
model=changeRxnBounds(model,'R088', 0, 'b');%
%model=changeRxnBounds(model,'R450', 0, 'b');%can't remove
model=changeRxnBounds(model,'R307', 0, 'b');%
model=changeRxnBounds(model,'R380', 0, 'b');%
% need to be removed to make 13PDO essential but get redundant after
R104
% removal
%model=changeRxnBounds(model,'R100', 0, 'b');%
model=changeRxnBounds(model,'R333', 0, 'b');%remove
model=changeRxnBounds(model,'R246', 0, 'b');%remove
model=changeRxnBounds(model,'R336', 0, 'b');%remove
%model=changeRxnBounds(model,'R104', 0, 'b');
```

```
%}
%[a,b]=fluxVariability(model,100,'max',{'EX_592'}, false, true);
%beta oxidation cycle reversal
% thiolase - 1
%
model = addReaction(model,'BR01', 'ACCOA + ACAC -> AACOA +
AC');%acetoacetyl-CoA transferase
model = addReaction(model,'BR02', '2 ACCOA <=> AACOA + COA');%acetyl-
CoA C-acetyltransferase
model = addReaction(model,'BR03', 'ACCOA + BTCOA <=> 3OHCOA +
COA');%acetyl-CoA C-acyltransferase (butanoyl-CoA)
model = addReaction(model,'BR04', 'ACCOA + HXCOA <=> 3OOCOA +
COA');%acetyl-CoA C-acyltransferase (hexanoyl-CoA)
model = addReaction(model,'BR05', 'ACCOA + OCCOA <=> 3ODCOA +
COA');%acetyl-CoA C-acyltransferase (octanoyl-CoA)
model = addReaction(model,'BR06', 'ACCOA + DCACOA <=> 3ODDCOA +
COA');%acetyl-CoA C-acyltransferase (decanoyl-CoA)
model = addReaction(model,'BR07', 'ACCOA + DDCACOA <=> 3OTDCOA +
COA');%acetyl-CoA C-acyltransferase (dodecanoyl-CoA)
model = addReaction(model,'BR08', 'ACCOA + TDCOA <=> 3OHDCOA +
COA');%acetyl-CoA C-acyltransferase (tetradecanoyl-CoA)
model = addReaction(model,'BR09', 'ACCOA + PMTCOA <=> 3OODCOA +
COA');%acetyl-CoA acyltransferase (hexadecanoyl-CoA)
% hydroxyacyl coa dehydrogenase - 2
model = addReaction(model,'BR10', 'AACOA + NADH + H <=> 3HBCOA +
NAD');%3-hydroxyacyl-CoA dehydrogenase (acetoacetyl-CoA)%A instead of C
model = addReaction(model,'BR11', '3OHCOA + NADH + H <=> 3HHCOA +
NAD');%3-hydroxyacyl-CoA dehydrogenase (3-oxohexanoyl-CoA)
model = addReaction(model,'BR12', '3OOCOA + NADH + H <=> 3HOCOA +
NAD');%3-hydroxyacyl-CoA dehydrogenase (3-oxooctanoyl-CoA)
model = addReaction(model,'BR13', '3ODCOA + NADH + H <=> 3HDCOA +
NAD');%3-hydroxyacyl-CoA dehydrogenase (3-oxodecanoyl-CoA)
model = addReaction(model,'BR14', '3ODDCOA + NADH + H <=> 3HDDCOA +
NAD');%3-hydroxyacyl-CoA dehydrogenase (3-oxododecanoyl-CoA)
model = addReaction(model,'BR15', '3OTDCOA + NADH + H <=> 3HTDCOA +
NAD');%3-hydroxyacyl-CoA dehydrogenase (3-oxotetradecanoyl-CoA)
model = addReaction(model,'BR16', '3OHDCOA + NADH + H <=> 3HHDCOA +
NAD');%3-hydroxyacyl-CoA dehydrogenase (3-oxohexadecanoyl-CoA)
model = addReaction(model,'BR17', '3OODCOA + NADH + H <=> 3HODCOA +
NAD');%3-hydroxyacyl-CoA dehydrogenase (3-oxooctadecanoyl-CoA),
peroxisomal
% enoyl-CoA hydratase - 3
model = addReaction(model,'BR18', '3HBCOA <=> B2COA');%3-hydroxyacyl-
CoA dehydratase (3-hydroxybutanoyl-CoA)
model = addReaction(model,'BR19', '3HHCOA <=> HX2COA');%3-hydroxyacyl-
CoA dehydratase (3-hydroxyhexanoyl-CoA)
model = addReaction(model,'BR20', '3HOCOA <=> OC2COA');%3-hydroxyacyl-
CoA dehydratase (3-hydroxyoctanoyl-CoA)
model = addReaction(model,'BR21', '3HDCOA <=> DC2COA');%3-hydroxyacyl-
CoA dehydratase (3-hydroxydecanoyl-CoA)
model = addReaction(model,'BR22', '3HDDCOA <=> DD2COA');%3-hydroxyacyl-
CoA dehydratase (3-hydroxydodecanoyl-CoA)
model = addReaction(model,'BR23', '3HTDCOA <=> TD2COA');%3-hydroxyacyl-
CoA dehydratase (3-hydroxytetradecanoyl-CoA)
```

```
model = addReaction(model,'BR24', '3HHDCOA <=> HDD2COA');%3-
hydroxyacyl-CoA dehydratase (3-hydroxyhexadecanoyl-CoA)
model = addReaction(model,'BR25', '3HODCOA <=> OD2COA');%3-hydroxyacyl-
CoA dehydratase (3-hydroxyoctadecanoyl-CoA)
%enoyl-coa reductase(FadE)  - 4
    %{
model = addReaction(model,'BR26', 'BTCOA + FAD <=> B2COA +
FADH2');%acyl-CoA dehydrogenase (butanoyl-CoA)
model = addReaction(model,'BR27', 'HXCOA + FAD <=> HX2COA +
FADH2');%acyl-CoA dehydrogenase (hexanoyl-CoA)
model = addReaction(model,'BR28', 'OCCOA + FAD <=> OC2COA +
FADH2');%acyl-CoA dehydrogenase (octanoyl-CoA)
model = addReaction(model,'BR29', 'DCACOA + FAD <=> DC2COA +
FADH2');%acyl-CoA dehydrogenase (decanoyl-CoA)
model = addReaction(model,'BR30', 'DDCACOA + FAD <=> DD2COA +
FADH2');%acyl-CoA dehydrogenase (dodecanoyl-CoA)
model = addReaction(model,'BR31', 'TDCOA + FAD <=> TD2COA +
FADH2');%acyl-CoA dehydrogenase (tetradecanoyl-CoA)
model = addReaction(model,'BR32', 'PMTCOA + FAD <=> HDD2COA +
FADH2');%acyl-CoA dehydrogenase (hexadecanoyl-CoA)
model = addReaction(model,'BR33', 'STCOA + FAD <=> OD2COA +
FADH2');%acyl-CoA dehydrogenase (octadecanoyl-CoA)
    %}
%enoyl-coa reductase(egTER)  - 4
%
model = addReaction(model,'BR34', 'BTCOA + NAD <=> B2COA + NADH +
H');%acyl-CoA dehydrogenase (butanoyl-CoA)
model = addReaction(model,'BR35', 'HXCOA + NAD <=> HX2COA + NADH +
H');%acyl-CoA dehydrogenase (hexanoyl-CoA)
model = addReaction(model,'BR36', 'OCCOA + NAD <=> OC2COA + NADH +
H');%acyl-CoA dehydrogenase (octanoyl-CoA)
model = addReaction(model,'BR37', 'DCACOA + NAD <=> DC2COA + NADH +
H');%acyl-CoA dehydrogenase (decanoyl-CoA)
model = addReaction(model,'BR38', 'DDCACOA + NAD <=> DD2COA + NADH +
H');%acyl-CoA dehydrogenase (dodecanoyl-CoA)
model = addReaction(model,'BR39', 'TDCOA + NAD <=> TD2COA + NADH +
H');%acyl-CoA dehydrogenase (tetradecanoyl-CoA)
model = addReaction(model,'BR40', 'PMTCOA + NAD <=> HDD2COA + NADH +
H');%acyl-CoA dehydrogenase (hexadecanoyl-CoA)
model = addReaction(model,'BR41', 'STCOA + NAD <=> OD2COA + NADH +
H');%acyl-CoA dehydrogenase (octadecanoyl-CoA)
%}
%% termination after step 4
%acids
%{
model = addReaction(model,'BR42', 'BTCOA <=> BTA + COA + H');%fatty-
acid-CoA thioesterase (butanoate)
model = addReaction(model,'BR43', 'BTA + H -> HEXT');
%
model = addReaction(model,'BR44', 'HXCOA <=> HXA + COA + H');%fatty-
acid-CoA thioesterase (hexanoate)
model = addReaction(model,'BR45', 'HXA + H -> HEXT');
%
model = addReaction(model,'BR46', 'OCCOA <=> OCTA + COA + H');%fatty-
acid-CoA thioesterase (octanoate)
```

```
model = addReaction(model,'BR61', 'OCTA + H -> HEXT');
%
model = addReaction(model,'BR47', 'DCACOA <=> DCA + COA + H');%fatty-
acid-CoA thioesterase (decanoate)
model = addReaction(model,'BR48', 'DCA + H -> HEXT');
%
model = addReaction(model,'BR49', 'DDCACOA <=> DDCA + COA + H');%fatty-
acid-CoA thioesterase (dodecanoate)
model = addReaction(model,'BR50', 'DDCA + H -> HEXT');
%
model = addReaction(model,'BR51', 'TDCOA <=> TTDCA + COA + H');%fatty-
acid-CoA thioesterase (tetradecanoate)
model = addReaction(model,'BR52', 'TTDCA + H -> HEXT');
%
model = addReaction(model,'BR53', 'PMTCOA <=> HDCA + COA + H');%fatty-
acid-CoA thioesterase (hexadecanoate)
model = addReaction(model,'BR54', 'HDCA + H -> HEXT');
%
model = addReaction(model,'BR55', 'STCOA <=> OCDCA + COA + H');%fatty-
acid-CoA thioesterase (octadecanoate)
model = addReaction(model,'BR56', 'OCDCA + H -> HEXT');
%}
%alcohols
%{
model = addReaction(model,'BR62', 'BTCOA + NADH + H <=> BUTAL + NAD +
COA');%acyl-CoA reductase (butanal)
model = addReaction(model,'BR63', '1BOH + H -> HEXT');
%
model = addReaction(model,'BR64', 'HXCOA + NADH + H <=> HXAL + NAD +
COA');%acyl-CoA reductase (hexanal)
model = addReaction(model,'BR65', 'HXAL + NADH + H <=> HXOH + NAD');
model = addReaction(model,'BR66', 'HXOH + H -> HEXT');
%
model = addReaction(model,'BR67', 'OCCOA + NADH + H <=> OCAL + NAD +
COA');%acyl-CoA reductase (octanal)
model = addReaction(model,'BR68', 'OCAL + NADH + H <=> OCOH + NAD');
model = addReaction(model,'BR69', 'OCOH + H -> HEXT');
%
model = addReaction(model,'BR70', 'DCACOA + NADH + H <=> DCAAL + NAD +
COA');%acyl-CoA reductase (decaanal)
model = addReaction(model,'BR71', 'DCAAL + NADH + H <=> DCAOH + NAD');
model = addReaction(model,'BR72', 'DCAOH + H -> HEXT');
%
model = addReaction(model,'BR73', 'DDCACOA + NADH + H <=> DDCAAL + NAD
+ COA');%acyl-CoA reductase (dodecanal)
model = addReaction(model,'BR74', 'DDCAAL + NADH + H <=> DDCAOH +
NAD');
model = addReaction(model,'BR75', 'DDCAOH + H -> HEXT');
%
model = addReaction(model,'BR76', 'TDCOA + NADH + H <=> TDAL + NAD +
COA');%acyl-CoA reductase (tetradecanal)
model = addReaction(model,'BR77', 'TDAL + NADH + H <=> TDOH + NAD');
model = addReaction(model,'BR78', 'TDOH + H -> HEXT');
%
```

```
model = addReaction(model,'BR79', 'PMTCOA + NADH + H <=> PMTAL + NAD +
COA');%acyl-CoA reductase (hexadecanal)
model = addReaction(model,'BR80', 'PMTAL + NADH + H <=> PMTOH + NAD');
model = addReaction(model,'BR81', 'PMTOH + H -> HEXT');
%
model = addReaction(model,'BR82', 'STCOA + NADH + H <=> STAL + NAD +
COA');%acyl-CoA reductase (octadecanal)
model = addReaction(model,'BR83', 'STAL + NADH + H <=> STOH + NAD');
model = addReaction(model,'BR84', 'STOH + H -> HEXT');
%}
%alkanes
%{
model = addReaction(model,'BR85', 'PROP + CO <=> BUTAL');%aldehyde
decarbonylase (propane)
model = addReaction(model,'BR86', 'PROP ->');
%
model = addReaction(model,'BR87', 'PEN + CO <=> HXAL');%aldehyde
decarbonylase (pentane)
model = addReaction(model,'BR88', 'PEN ->');
%
model = addReaction(model,'BR89', 'HEP + CO <=> OCAL');%aldehyde
decarbonylase (heptane)
model = addReaction(model,'BR90', 'HEP ->');
%
model = addReaction(model,'BR91', 'NON + CO <=> DCAAL');%aldehyde
decarbonylase (nonane)
model = addReaction(model,'BR92', 'NON ->');
%
model = addReaction(model,'BR93', 'UND + CO <=> DDCAAL');%aldehyde
decarbonylase (undecane)
model = addReaction(model,'BR94', 'UND ->');
%
model = addReaction(model,'BR95', 'TRD + CO <=> TDAL');%aldehyde
decarbonylase (tridecane)
model = addReaction(model,'BR96', 'TRD ->');
%
model = addReaction(model,'BR97', 'PND + CO <=> PMTAL');%aldehyde
decarbonylase (pentadecane)
model = addReaction(model,'BR98', 'PND ->');
%
model = addReaction(model,'BR99', 'HPD + CO <=> STAL');%aldehyde
decarbonylase (heptadecane)
model = addReaction(model,'BR100', 'HPD ->');
%
model = addReaction(model,'BR101', 'CO ->');
model=changeRxnBounds(model,'BR63', 0, 'b');%Butanol
model=changeRxnBounds(model,'BR66', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR69', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR72', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR75', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR78', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR81', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR84', 0, 'b');%Octadecanol
%}
%% termination after step 3
```

```
%enoyl acids
%{
model = addReaction(model,'BR102', 'B2COA <=> B2A + COA + H');%fatty-
acid-CoA thioesterase (butanoate)% no growth
model = addReaction(model,'BR103', 'B2A + H -> HEXT');
%
model = addReaction(model,'BR106', 'HX2COA <=> HX2A + COA + H');%fatty-
acid-CoA thioesterase (hexanoate)
model = addReaction(model,'BR107', 'HX2A + H -> HEXT');
%
model = addReaction(model,'BR108', 'OC2COA <=> OCT2A + COA +
H');%fatty-acid-CoA thioesterase (octanoate)
model = addReaction(model,'BR109', 'OCT2A + H -> HEXT');
%
model = addReaction(model,'BR110', 'DC2COA <=> DC2A + COA + H');%fatty-
acid-CoA thioesterase (decanoate)
model = addReaction(model,'BR111', 'DC2A + H -> HEXT');
%
model = addReaction(model,'BR112', 'DD2COA <=> DD2A + COA + H');%fatty-
acid-CoA thioesterase (dodecanoate)
model = addReaction(model,'BR113', 'DD2A + H -> HEXT');
%
model = addReaction(model,'BR114', 'TD2COA <=> TTD2CA + COA +
H');%fatty-acid-CoA thioesterase (tetradecanoate)
model = addReaction(model,'BR115', 'TTD2CA + H -> HEXT');
%
model = addReaction(model,'BR116', 'HDD2COA <=> HD2CA + COA +
H');%fatty-acid-CoA thioesterase (hexadecanoate)
model = addReaction(model,'BR117', 'HD2CA + H -> HEXT');
%
model = addReaction(model,'BR118', 'OD2COA <=> OCD2CA + COA +
H');%fatty-acid-CoA thioesterase (octadecanoate)
model = addReaction(model,'BR119', 'OCD2CA + H -> HEXT');
%}
%enoyl alcohols
%{
model = addReaction(model,'BR122', 'B2COA + NADH + H <=> BUT2AL + NAD +
COA');%acyl-CoA reductase (butanal)
model = addReaction(model,'BR123', 'BUT2AL + NADH + H <=> B2OH + NAD');
model = addReaction(model,'BR124', 'B2OH + H -> HEXT');
%
model = addReaction(model,'BR125', 'HX2COA + NADH + H <=> HX2AL + NAD +
COA');%acyl-CoA reductase (hexanal)
model = addReaction(model,'BR126', 'HX2AL + NADH + H <=> HX2OH + NAD');
model = addReaction(model,'BR127', 'HX2OH + H -> HEXT');
%
model = addReaction(model,'BR128', 'OC2COA + NADH + H <=> OC2AL + NAD +
COA');%acyl-CoA reductase (octanal)
model = addReaction(model,'BR129', 'OC2AL + NADH + H <=> OC2OH + NAD');
model = addReaction(model,'BR130', 'OC2OH + H -> HEXT');
%
model = addReaction(model,'BR131', 'DC2COA + NADH + H <=> DC2AL + NAD +
COA');%acyl-CoA reductase (decaanal)
model = addReaction(model,'BR132', 'DC2AL + NADH + H <=> DC2OH + NAD');
model = addReaction(model,'BR133', 'DC2OH + H -> HEXT');
```

```
%
model = addReaction(model,'BR134', 'DD2COA + NADH + H <=> DD2AL + NAD +
COA');%acyl-CoA reductase (dodecanal)
model = addReaction(model,'BR135', 'DD2AL + NADH + H <=> DD2OH + NAD');
model = addReaction(model,'BR136', 'DD2OH + H -> HEXT');
%
model = addReaction(model,'BR137', 'TD2COA + NADH + H <=> TD2AL + NAD +
COA');%acyl-CoA reductase (tetradecanal)
model = addReaction(model,'BR138', 'TD2AL + NADH + H <=> TD2OH + NAD');
model = addReaction(model,'BR139', 'TD2OH + H -> HEXT');
%
model = addReaction(model,'BR140', 'HDD2COA + NADH + H <=> HDD2AL + NAD
+ COA');%acyl-CoA reductase (hexadecanal)
model = addReaction(model,'BR141', 'HDD2AL + NADH + H <=> HDD2OH +
NAD');
model = addReaction(model,'BR142', 'HDD2OH + H -> HEXT');
%
model = addReaction(model,'BR143', 'OD2COA + NADH + H <=> OD2AL + NAD +
COA');%acyl-CoA reductase (octadecanal)
model = addReaction(model,'BR144', 'OD2AL + NADH + H <=> OD2OH + NAD');
model = addReaction(model,'BR145', 'OD2OH + H -> HEXT');
%}
%alkenes
%{
model = addReaction(model,'BR146', 'PROP2 + CO <=> BUT2AL');%aldehyde
decarbonylase (propene)
model = addReaction(model,'BR147', 'PROP2 ->');
%
model = addReaction(model,'BR148', 'PEN2 + CO <=> HX2AL');%aldehyde
decarbonylase (pentene)
model = addReaction(model,'BR149', 'PEN2 ->');
%
model = addReaction(model,'BR150', 'HEP2 + CO <=> OC2AL');%aldehyde
decarbonylase (heptene)
model = addReaction(model,'BR151', 'HEP2 ->');
%
model = addReaction(model,'BR152', 'NON2 + CO <=> DC2AL');%aldehyde
decarbonylase (nonene)
model = addReaction(model,'BR153', 'NON2 ->');
%
model = addReaction(model,'BR154', 'UND2 + CO <=> DD2AL');%aldehyde
decarbonylase (undecene)
model = addReaction(model,'BR155', 'UND2 ->');
%
model = addReaction(model,'BR156', 'TRD2 + CO <=> TD2AL');%aldehyde
decarbonylase (tridecene)
model = addReaction(model,'BR157', 'TRD2 ->');
%
model = addReaction(model,'BR158', 'PND2 + CO <=> HDD2AL');%aldehyde
decarbonylase (pentadecene)
model = addReaction(model,'BR159', 'PND2 ->');
%
model = addReaction(model,'BR160', 'HPD2 + CO <=> OD2AL');%aldehyde
decarbonylase (heptadecene)
model = addReaction(model,'BR161', 'HPD2 ->');
```

```
%
model = addReaction(model,'BR162', 'CO ->');
model=changeRxnBounds(model,'BR124', 0, 'b');%Butanol
model=changeRxnBounds(model,'BR127', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR130', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR133', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR136', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR139', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR142', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR145', 0, 'b');%Octadecanol
%}
%% termination after step 2
%hydroxy acids
%{
model = addReaction(model,'BR171', '3HBCOA <=> 3HBA + COA + H');%fatty-
acid-CoA thioesterase (butanoate)- no growth
model = addReaction(model,'BR172', '3HBA + H -> HEXT');
%
model = addReaction(model,'BR177', '3HHCOA <=> 3HHA + COA + H');%fatty-
acid-CoA thioesterase (hexanoate)
model = addReaction(model,'BR179', '3HHA + H -> HEXT');
%
model = addReaction(model,'BR180', '3HOCOA <=> 3HOA + COA + H');%fatty-
acid-CoA thioesterase (octanoate)
model = addReaction(model,'BR181', '3HOA + H -> HEXT');
%
model = addReaction(model,'BR163', '3HDCOA <=> 3HDA + COA + H');%fatty-
acid-CoA thioesterase (decanoate)
model = addReaction(model,'BR164', '3HDA + H -> HEXT');
%
model = addReaction(model,'BR165', '3HDDCOA <=> 3HDDA + COA +
H');%fatty-acid-CoA thioesterase (dodecanoate)
model = addReaction(model,'BR166', '3HDDA + H -> HEXT');
%
model = addReaction(model,'BR167', '3HTDCOA <=> 3HTDCA + COA +
H');%fatty-acid-CoA thioesterase (tetradecanoate)
model = addReaction(model,'BR168', '3HTDCA + H -> HEXT');
%
model = addReaction(model,'BR169', '3HHDCOA <=> 3HHCA + COA +
H');%fatty-acid-CoA thioesterase (hexadecanoate)
model = addReaction(model,'BR170', '3HHCA + H -> HEXT');
%}
model = addReaction(model,'BR173', '3HODCOA <=> 3HODCA + COA +
H');%fatty-acid-CoA thioesterase (octadecanoate)
model = addReaction(model,'BR174', '3HODCA + H -> HEXT');
%}
%hydroxy alcohols
%{
model = addReaction(model,'BR182', '3HBCOA + NADH + H <=> 3HBAL + NAD +
COA');%acyl-CoA reductase (butanal)%no growth
model = addReaction(model,'BR183', '3HBAL + NADH + H <=> 3HBOH + NAD');
model = addReaction(model,'BR184', '3HBOH + H -> HEXT');
%
model = addReaction(model,'BR185', '3HHCOA + NADH + H <=> 3HHAL + NAD +
COA');%acyl-CoA reductase (hexanal)
```

```
model = addReaction(model,'BR186', '3HHAL + NADH + H <=> 3HHOH + NAD');
model = addReaction(model,'BR187', '3HHOH + H -> HEXT');
%
model = addReaction(model,'BR188', '3HOCOA + NADH + H <=> 3HOAL + NAD +
COA');%acyl-CoA reductase (octanal)
model = addReaction(model,'BR189', '3HOAL + NADH + H <=> 3HOOH + NAD');
model = addReaction(model,'BR190', '3HOOH + H -> HEXT');
%
model = addReaction(model,'BR191', '3HDCOA + NADH + H <=> 3HDAL + NAD +
COA');%acyl-CoA reductase (decaanal)
model = addReaction(model,'BR192', '3HDAL + NADH + H <=> 3HDOH + NAD');
model = addReaction(model,'BR193', '3HDOH + H -> HEXT');
%
model = addReaction(model,'BR194', '3HDDCOA + NADH + H <=> 3HDDAL + NAD
+ COA');%acyl-CoA reductase (dodecanal)
model = addReaction(model,'BR195', '3HDDAL + NADH + H <=> 3HDDOH +
NAD');
model = addReaction(model,'BR196', '3HDDOH + H -> HEXT');
%
model = addReaction(model,'BR197', '3HTDCOA + NADH + H <=> 3HTAL + NAD
+ COA');%acyl-CoA reductase (tetradecanal)
model = addReaction(model,'BR198', '3HTAL + NADH + H <=> 3HTOH + NAD');
model = addReaction(model,'BR199', '3HTOH + H -> HEXT');
%
model = addReaction(model,'BR200', '3HHDCOA + NADH + H <=> 3HHDAL + NAD
+ COA');%acyl-CoA reductase (hexadecanal)
model = addReaction(model,'BR201', '3HHDAL + NADH + H <=> 3HHDOH +
NAD');
model = addReaction(model,'BR202', '3HHDOH + H -> HEXT');
%
model = addReaction(model,'BR203', '3HODCOA + NADH + H <=> 3HODAL + NAD
+ COA');%acyl-CoA reductase (octadecanal)
model = addReaction(model,'BR204', '3HODAL + NADH + H <=> 3HODOH +
NAD');
model = addReaction(model,'BR205', '3HODOH + H -> HEXT');
%}
%hydroxy alkanes
%{
model = addReaction(model,'BR206', '3HPROP + CO <=> 3HBAL');%aldehyde
decarbonylase (propene)
model = addReaction(model,'BR207', '3HPROP ->');
%
model = addReaction(model,'BR208', '3HPEN + CO <=> 3HHAL');%aldehyde
decarbonylase (pentene)
model = addReaction(model,'BR209', '3HPEN ->');
%
model = addReaction(model,'BR210', '3HHEP + CO <=> 3HOAL');%aldehyde
decarbonylase (heptene)
model = addReaction(model,'BR211', '3HHEP ->');
%
model = addReaction(model,'BR212', '3HNON + CO <=> 3HDAL');%aldehyde
decarbonylase (nonene)
model = addReaction(model,'BR213', '3HNON ->');
%
```

```
model = addReaction(model,'BR214', '3HUND + CO <=> 3HDDAL');%aldehyde
decarbonylase (undecene)
model = addReaction(model,'BR215', '3HUND ->');
%
model = addReaction(model,'BR216', '3HTRD + CO <=> 3HTAL');%aldehyde
decarbonylase (tridecene)
model = addReaction(model,'BR217', '3HTRD ->');
%
model = addReaction(model,'BR218', '3HPND + CO <=> 3HHDAL');%aldehyde
decarbonylase (pentadecene)
model = addReaction(model,'BR219', '3HPND ->');
%
model = addReaction(model,'BR220', '3HHPD + CO <=> 3HODAL');%aldehyde
decarbonylase (heptadecene)
model = addReaction(model,'BR221', '3HHPD ->');
%
model = addReaction(model,'BR222', 'CO ->');
model=changeRxnBounds(model,'BR184', 0, 'b');%Butanol
model=changeRxnBounds(model,'BR187', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR190', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR193', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR196', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR199', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR202', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR205', 0, 'b');%Octadecanol

%}
%% termination after step 1
%ACLY COA thioesterase-UNSTABLE
%oxo-alcohols
%{
model = addReaction(model,'BR223', 'AACOA + NADH + H <=> AAAL + NAD +
COA');%acyl-CoA reductase (butanal)
model = addReaction(model,'BR224', 'AAAL + NADH + H <=> AAOH + NAD');
model = addReaction(model,'BR225', 'AAOH + H -> HEXT');
%
model = addReaction(model,'BR226', '3OHCOA + NADH + H <=> 3OHAL + NAD +
COA');%acyl-CoA reductase (hexanal)
model = addReaction(model,'BR227', '3OHAL + NADH + H <=> 3OHOH + NAD');
model = addReaction(model,'BR228', '3OHOH + H -> HEXT');
%
model = addReaction(model,'BR229', '3OOCOA + NADH + H <=> 3OOAL + NAD +
COA');%acyl-CoA reductase (octanal)
model = addReaction(model,'BR230', '3OOAL + NADH + H <=> 3OOOH + NAD');
model = addReaction(model,'BR231', '3OOOH + H -> HEXT');
%
model = addReaction(model,'BR232', '3ODCOA + NADH + H <=> 3ODAL + NAD +
COA');%acyl-CoA reductase (decaanal)
model = addReaction(model,'BR233', '3ODAL + NADH + H <=> 3ODOH + NAD');
model = addReaction(model,'BR234', '3ODOH + H -> HEXT');
%
model = addReaction(model,'BR235', '3ODDCOA + NADH + H <=> 3ODDAL + NAD
+ COA');%acyl-CoA reductase (dodecanal)
model = addReaction(model,'BR236', '3ODDAL + NADH + H <=> 3ODDOH +
NAD');
```

```
model = addReaction(model,'BR237', '3ODDOH + H -> HEXT');
%
model = addReaction(model,'BR238', '3OTDCOA + NADH + H <=> 3OTDAL + NAD
+ COA');%acyl-CoA reductase (tetradecanal)
model = addReaction(model,'BR239', '3OTDAL + NADH + H <=> 3OTDOH +
NAD');
model = addReaction(model,'BR240', '3OTDOH + H -> HEXT');
%
model = addReaction(model,'BR241', '3OHDCOA + NADH + H <=> 3OHDAL + NAD
+ COA');%acyl-CoA reductase (hexadecanal)
model = addReaction(model,'BR242', '3OHDAL + NADH + H <=> 3OHDOH +
NAD');
model = addReaction(model,'BR243', '3OHDOH + H -> HEXT');
%
model = addReaction(model,'BR244', '3OODCOA + NADH + H <=> 3OODAL + NAD
+ COA');%acyl-CoA reductase (octadecanal)
model = addReaction(model,'BR245', '3OODAL + NADH + H <=> 3OODOH +
NAD');
model = addReaction(model,'BR246', '3OODOH + H -> HEXT');
%}
%oxo-alkanes
%{
model = addReaction(model,'BR247', '3OPROP + CO <=> AAAL');%aldehyde
decarbonylase (propene)
model = addReaction(model,'BR248', '3OPROP ->');
%
model = addReaction(model,'BR249', '3OPEN + CO <=> 3OHAL');%aldehyde
decarbonylase (pentene)
model = addReaction(model,'BR250', '3OPEN ->');
%
model = addReaction(model,'BR251', '3OHEP + CO <=> 3OOAL');%aldehyde
decarbonylase (heptene)
model = addReaction(model,'BR252', '3OHEP ->');
%
model = addReaction(model,'BR253', '3ONON + CO <=> 3ODAL');%aldehyde
decarbonylase (nonene)
model = addReaction(model,'BR254', '3ONON ->');
%
model = addReaction(model,'BR255', '3OUND + CO <=> 3ODDAL');%aldehyde
decarbonylase (undecene)
model = addReaction(model,'BR256', '3OUND ->');
%
model = addReaction(model,'BR257', '3OTRD + CO <=> 3OTDAL');%aldehyde
decarbonylase (tridecene)
model = addReaction(model,'BR258', '3OTRD ->');
%
model = addReaction(model,'BR259', '3OPND + CO <=> 3OHDAL');%aldehyde
decarbonylase (pentadecene)
model = addReaction(model,'BR260', '3OPND ->');
%
model = addReaction(model,'BR261', '3OHPD + CO <=> 3OODAL');%aldehyde
decarbonylase (heptadecene)
model = addReaction(model,'BR262', '3OHPD ->');
%
model = addReaction(model,'BR263', 'CO ->');
```

```
model=changeRxnBounds(model,'BR225', 0, 'b');%Butanol
model=changeRxnBounds(model,'BR228', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR231', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR234', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR237', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR240', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR243', 0, 'b');%Fructose
model=changeRxnBounds(model,'BR246', 0, 'b');%Octadecanol
%}
model=changeRxnBounds(model,'R210', 0, 'b');%oroa
model = addReaction(model,'R210_1', 'DOROA + FUM <=> SUCC + OROA');
solution = optimizeCbModel(model)
[az,bz]=fluxVariability(model,100,'max',{'BR174'}, false, true);
%solution space
%
solution = optimizeCbModel(model)
bio = 0.1512;
for i= 1:100
model=changeRxnBounds(model,'R601', (1-i/100)*bio , 'b');
[mi(100-i+1),ma(i)]=fluxVariability(model,100,'max',{'BR174'}, false,
true);
bi(i)=(1-i/100)*bio;
ibi(i)=bio*i/100;
end
m=[mi,ma];
b=[ibi,bi];
plot (b,m,'k')
hold on
title('Coupling of cell growth & alkenoic acid production using
xylose/arabinose','fontsize',20,'fontweight','b')
xlabel('Cell growth(Biomass) in
mmol/gDW/h','fontsize',18,'fontweight','b')
ylabel('alkenoic acid concentration in
mmol/gDW/h','fontsize',18,'fontweight','b')
legend('hexenoic acid','octenoic acid','decenoic acid','dodecenoic
acid','tetradecenoic acid','hexadecenoic acid','octadecenoic acid')
%}
```